THE ROLE OF DUNG BEETLES IN THE REGENERATION OF RAINFOREST
PLANTS IN CENTRAL AMAZONIA

By

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To my parents and sisters.

To my husband.

To Michifuz and Carlotta.

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Seed dispersal is an important process in the life history of many plant species.

Successful seed dispersal encompasses a series of stages, beginning with the removal of

the fruits and seeds from the parent tree and ending in seedling establishment. Yet,

studies on seed dispersal ecology generally do not link these stages.

Among the factors that can affect the fate of seeds after initial deposition, secondary

seed dispersal has received the least attention. Secondary seed dispersal is the movement

of seeds away from the initial site of deposition, and it can play an important role in

determining whether a seed survives to establish as a seedling. For example, when

primary dispersal occurs through defecation by mammals, the fecal material that

accompanies the seed rain attracts dung beetles that can act as secondary dispersers for

the defecated seeds. This can have a great effect on the fate of the seeds. The main

objective of this study was to link primary dispersal to seedling establishment, by determining the role that dung beetles play as secondary dispersers of seeds defecated by frugivorous mammals in a rainforest in Central Amazonia.

I found that secondary dispersal of seeds by dung beetles was affected by seed size, beetle size, time of day, season, and amount of dung surrounding the seeds. In terms of seedling establishment I found that the presence of dung around seeds had no effect on seedling establishment: the same percentage of seedlings established from seeds with no dung as from seeds surrounded by dung. However, for seeds surrounded by dung, I found that the probability of seedling establishment was much higher for seeds that had been buried by dung beetles than for seeds that remained on the surface.

Finally, I found that forest fragmentation can affect the interaction between seeds, seed predators, and secondary seed dispersers. In particular, seeds were buried less often in forest fragments, and seed predation was higher in forest fragments than in continuous forest. Some implications of these results for the conservation and management of tropical forests are discussed.

CHAPTER 1
GENERAL INTRODUCTION

The Ecological Perspective

It is widely recognized that seed dispersal is an important process in the life history

of many plant species, because it is beneficial for seed survival and seedling

establishment (see reviews in Howe and Smallwood 1982, Dirzo and Domínguez 1986,

Howe 1990, Willson 1992).

A crucial aspect in seed dispersal ecology, and one that has been relatively little

studied, is the fate of seeds between the stages of seed deposition and seed germination

(Chapman 1989, Levey and Byrne 1993, Adler and Kestell 1998, Andresen 1999).

Factors that affect the fate of seeds during this period, will ultimately determine whether

the fitness of a plant has or has not been increased by the primary seed dispersal event

(Coates-Estrada and Estrada 1988, Herrera et al. 1994). Thus, to better understand a

given fruit-frugivore interaction one needs to assess the fate of seeds from initial

deposition until at least the seedling establishment stage, and when possible even follow

the survival and growth of seedlings for several years (Herrera et al. 1994, Jordano and

Herrera 1995, Schupp and Fuentes 1995).

Among the factors that can affect the fate of dispersed seeds, secondary seed

dispersal has received the least attention. Secondary seed dispersal is the movement of

seeds away from the initial site of deposition by the primary dispersal agent, and it is

becoming increasingly apparent that secondary seed dispersal occurs in many dispersal systems (Böhning-Gaese et al. 1999). Consequently, to fully understand the regeneration process, it becomes important to assess how secondary dispersers affect the fate of seeds. The main objective of this study was to determine how a group of secondary dispersers affects the regeneration of plants that have their seeds dispersed by frugivorous mammals in a rainforest in Central Amazonia.

When primary dispersal occurs through defecation by mammals, the fecal material that accompanies the seed rain has the potential of affecting the fate of dispersed seeds. This aspect of mammalian seed dispersal has largely been neglected (but see Janzen 1986, Chapman 1989, Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999). Fecal material attracts seed predators (e.g., rodents) and secondary seed dispersers (e.g., dung beetles). Rodents actively search for seeds in dung and consume them. Dung beetles, on the other hand, do not feed on seeds but on the dung, with most species relocating portions of dung in burrows under the soil surface (Halffter and Edmonds 1982). In doing so they may incidentally move and relocate some of the seeds present in the dung. Thus, seed dispersal by beetles encompasses any horizontal and/or vertical movement of seeds away from the original place of deposition by the primary disperser.

The role that dung beetles play as secondary seed dispersers is poorly known. Only a few recent studies have started addressing this issue (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999, Vulinec 1999). The occurrence of secondary seed dispersal by dung beetles is likely to be affected by the types and numbers of dung beetles present in an area. Dung beetle communities can vary

tremendously from one geographic area to another (Hanski and Cambefort 1991b). Similarly, the assemblages of dung beetles attracted to dung within a forest can also vary greatly according to factors such as season and time of day (Hanski and Cambefort 1991b). Thus, it is necessary to study seed dispersal by dung beetles in different areas of the world and also under different circumstances in each area to gain a more complete understanding of how dung beetles affect regeneration of plants in tropical forests. The main objectives of CHAPTER 2 were to determine how several environmental factors affect the dung beetle assemblages attracted to dung, and to determine how these same factors, acting in concert with, seed size and beetle size affect the outcome of the seed-beetle interaction. In Chapter 2, I describe the dung beetle community in a rainforest in Central Amazonia and assess its role in secondary seed dispersal. I determine how time of day, season of the year, and amount of dung affect the species and numbers of dung beetles attracted to dung in a given day (dung beetle assemblage). I also quantify the percentage of seeds buried by dung beetles, as well as the horizontal and vertical distances at which seeds are relocated by dung beetles. Finally, I assess whether the factors mentioned above, as well as seed size and beetle size, affect secondary seed dispersal by dung beetles.

Variables related to primary dispersal, such as the spatial distribution of seeds and/or dung, may also affect the behavior of rodents and dung beetles and consequently seed fate. For example, a high density of dung and/or seeds may yield a stronger cue for rodents and dung beetles than a low density. Because frugivorous animals differ in the defecation patterns they produce (e.g., Andresen 1999), they may affect seed fate differently. Consequently, the defecation pattern of frugivorous animals is a characteristic

that should be considered when different primary dispersers are compared in terms of their seed dispersal quality for a given plant species. The main objective of CHAPTER 3, was to assess the effect of defecation pattern on short-term seed fate. In Chapter 3 I describe several aspects, such as fruit diet and defecation pattern, which are related to primary seed dispersal by howler monkeys (*Alouatta seniculus*) in Central Amazonia. First I quantify the number of species of plants dispersed by them and describe their defecation pattern in terms of dung and seed spatial distributions. Then, to assess whether defecation pattern has an effect on quality of dispersal conveyed by a given frugivore, I compare the short-term fate of seeds dispersed in a pattern mimicking dispersal by howler monkeys (high density of seeds and dung) with a scattered pattern mimicking dispersal by other frugivores such as smaller arboreal mammals (low density of seeds and dung)

Studies previously done on seed dispersal by dung beetles have shown that vertical secondary seed dispersal, i.e., seed burial, can greatly reduce the probability of seed predation by rodents (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999). Thus, it is generally assumed that the overall effect of dung beetles on plant regeneration is positive. However, germination experiments with seeds placed at different depths (Shepherd and Chapman 1998, Feer 1999) have suggested that seed burial by dung beetles may have a negative effect due to increased emergence failure of germinating seeds that are buried too deeply. Yet, it is still necessary to follow the fates of seeds dispersed through defecation by mammals until at least seedling establishment. This permits a more accurate assessment of the overall net effect of seed burial by dung beetles on plant regeneration. The main goal of CHAPTER 4 was to link

the primary seed dispersal stage (defecation of seeds by mammals) to the seedling stage, by assessing the interaction between seeds in dung, secondary dispersers and seed predators during the period between seed deposition and seedling establishment. In Chapter 4 I follow seeds of several Central Amazonian tree species until seedling establishment. I compare the fate of seeds in the following treatments: (i) seeds placed with different amounts of dung (to simulate seeds defecated by different mammal species) and either buried or not by dung beetles; and, (ii) seeds placed without dung (to simulate seeds dispersed by other means, e.g. regurgitated by birds, spit out by mammals). I quantify the depths at which seeds were buried by dung beetles, and conduct controlled germination experiments to determine the effect of seed burial-depth on seedling establishment.

The Conservation Perspective

The study of seed dispersal by animals is important for more than purely academic reasons; it can also have important implications for the management and conservation of natural areas, especially rainforests (Howe 1984, Pannell 1989, Terborgh 1990, Redford 1992, Chapman and Chapman 1995, Guariguata and Pinard 1998). Deforestation in tropical forests is occurring at an alarming rate, and biodiversity is greatly threatened by it, and by the resulting forest fragmentation (Harris 1984, Wilson 1988, Laurance and Bierregaard 1997). In tropical rainforests the majority of tree species produce fruits that appear adapted for seed dispersal by frugivorous animals (Howe 1990), which in turn dominate the vertebrate biomass in tropical forests (Terborgh 1986a). Although it has been often stated that habitat fragmentation is likely to disrupt plant-animal interactions,

very few studies have addressed this issue (Renner 1998). Thus, studies on the interaction between rainforest plants and their animal seed dispersers in forest fragments are urgently needed. By studying the effect of fragmentation on plant-animal interactions, we not only gain insight into how fragmentation affects the particular species involved in the interaction, but also into how the process of forest regeneration may be affected by this kind on disturbance. Only by understanding how this key process works can we make recommendation for the long-term conservation and management of fragmented forest landscapes.

The main objective of CHAPTER 5 was to assess whether the interaction described in the previous chapter between seeds in dung, secondary seed dispersers (dung beetles), and seed predators, is affected by forest fragmentation. In Chapter 5 I compare the seed-beetle-rodent interaction in continuous forest with that in isolated 1 ha and 10 ha forest fragments. In the three types of habitats I quantify the dung beetle community and assess seed burial by dung beetles as well as seed predation pressures. I also follow the fate of seeds until seedling establishment for three tree species in continuous forest and forest fragments. I conclude with implications of this study for the conservation and management of Neotropical forest fragments.

In summary this study contributes to the field of seed dispersal ecology by linking the stages of primary seed dispersal and seedling establishment, while taking into consideration the characteristics associated with seed depositon (presence of dung, amount of dung, defecation pattern). This study also contributes by stressing the importance of considering secondary seed dispersal, when studying the seed dispersal

ecology of a plant species. For the particular case of tropical rainforest, this study contributes to increasing the little knowledge we have regarding the ecological role of dung beetles as secondary seed dispersers. This is the first study that follows the fate of seeds dispersed by dung beetles until seedling establishment.

CHAPTER 2
DUNG BEETLES IN A CENTRAL AMAZONIAN RAINFOREST AND THEIR
BEHAVIOR AS SECONDARY SEED DISPERSERS

Introduction

Seed dispersal can be advantageous for plants because, through dispersal, seeds
may be deposited in sites in which the abiotic conditions (e.g., light and nutrients), and/or
the biotic conditions (e.g., low intraspecific competition, low predation) promote seed
survival and seedling establishment and growth (Howe and Smallwood 1982). Mostly,
the advantage conveyed by seed dispersal occurs during the seed and seedling stages.
Yet, most of the mortality in plants also occurs during these early stages (Harper 1977,
Fenner 1987, Terborgh 1990). Consequently, seed dispersal has the potential for greatly
increasing the fitness of a plant.

A very important stage in the seed dispersal process is the fate of seeds between the
stages of seed deposition and seedling establishment (Chapman 1989, Levey and Byrne
1993, Adler and Kestell 1998, Andresen 1999). This stage has received relatively little
attention from plant and animal ecologists, and yet, it is the biotic and abiotic factors that
affect the fate of seeds what will determine whether the fitness of a plant has been
affected by primary dispersal (Coates-Estrada and Estrada 1988, Herrera et al. 1994).

Biotic factors that can affect the fate of dispersed seeds include seed predation and
secondary seed dispersal. While seed predation has received more attention (e.g., De
Steven and Putz 1984, Boman and Casper 1995, Cintra and Horna 1997, Blate et al.

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1998, Forget et al. 1998), secondary seed dispersal has been the focus of relatively few studies. Secondary seed dispersal is the movement of seeds away from the initial site of deposition by the primary dispersal agent. Evidence is accumulating, however, showing that in many dispersal systems, seeds are dispersed not only by the primary disperser (e.g., wind, water, fruit-eating vertebrates), but also by secondary dispersers (e.g., rain, ants, rodents, Böhning-Gaese et al. 1999). Consequently, to better understand the dispersal ecology and thus the factors affecting fitness and regeneration of plants, it becomes important to assess how secondary dispersers affect the fate of seeds.

Several recent studies have shown that in tropical rainforests dung beetles can act as secondary dispersers of seeds that have been dispersed through mammal defecation (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999, Vulinec 1999). Mammal dung is nutritiously as well as quantitatively the most important resource for dung beetles in most regions (Hanski 1991). And since, at least in Neotropical rain forests, most mammal species include fruits in their diets (Terborgh 1986a), it is likely that much of the dung encountered by dung beetles in these forests will contain seeds.

Both adult and immature dung beetles feed on vertebrate feces. After locating a source of dung, the adults of most tropical dung beetle species rapidly relocate a portion of it, either for feeding or for oviposition (Halffter and Edmonds 1982, Hanski and Cambefort 1991b). Relocation of dung occurs mostly by burying it under the soil surface. Consequences of this dung-feeding and burying behavior include soil fertilization and aeration (Mittal 1993), an increase in the rate of nutrient cycling (Nealis 1977), and a reduced transmission of some parasites of vertebrates (Bergstrom et al. 1976). However,

very little is known about the ecological role that dung beetles play as secondary seed dispersers.

Dung beetles do not feed on seeds, but through the rapid relocation and burial of dung, they also relocate and bury some seeds, thus acting as secondary seed dispersers. Secondary seed dispersal by dung beetles can affect the survival probability of seeds and seedlings in several ways. Buried seeds are more likely to avoid detection by seed predators, than seeds on the surface (Johnson and Jorgensen 1981, Estrada and Coates-Estrada 1991, Crawley 1992, Shepherd and Chapman 1998, Andresen 1999, Feer 1999, Chapter 4). Seed burial may hinder seedling emergence in some plant species (Chapter 4). Buried seeds encounter a more humid environment than seeds on the surface; this may enhance seed survival and germination in many plant species, but it might also promote pathogen attack (Price and Jenkins 1986, Chambers and MacMahon 1994). Through their behavior, beetles may also move seeds horizontally (Shepherd and Chapman 1998, Andresen 1999, Feer 1999), thus decreasing the potentially negative effects of seed clumping and sibling competition (Howe 1989). Finally, the dung itself and/or the beetle's burying activity may promote germination and seedling establishment by aerating and fertilizing the soil (Mittal 1993) and by mulching (Coughenour and Detling 1986).

Whether a seed is secondarily dispersed by dung beetles seems to depend partly on the size of the seed (Fig 2.1). Since seeds in the dung constitute "contaminants" for dung beetles, larger seeds should be more frequently separated from the dung by the beetles, and consequently secondarily dispersed less often than smaller seeds. Previous studies have provided evidence supporting this (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999).
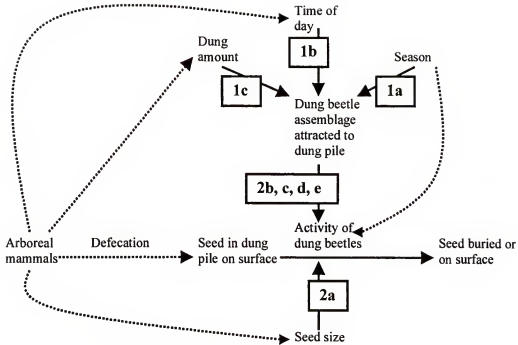
Figure 2.1 This diagram depicts the relationships between seeds, mammals and dung beetles, and some of the factors affecting the fate of seeds dispersed through mammal defecation. Solid arrows represent relationships addressed in this study, with the boxes on them indicating the number of the study's objective related to it. Dashed arrows indicate relationships not addressed in this study. Arboreal frugivores defecate seeds which end up on the forest floor surrounded by dung. The species of arboreal mammal will influence the size of the dung pile in which seeds are deposited, the time of day in which it is defecated, and to some degree also the size of the seed (in the sense that small mammals are not likely or able to defecate large seeds). Dung piles will be visited by dung beetles; dung beetle activity may result in the seed being buried or it remaining on the ground surface. The effect of dung beetle activity on seed-containing dung piles will be determined mostly by the size of the seed, and by the assemblage of dung beetles attracted to the dung pile. In turn, the composition of the dung beetle assemblage may be influenced by: dung amount, time of day (day vs. night) and season (dry vs. wet). Season can also directly affect dung beetle activity if, for example, the rain in some way facilitates burial of dung (e.g., by softening the soil).

On the other hand, the species and number of dung beetles attracted to a dung pile will likely also affect whether a seed in dung will be secondarily dispersed by dung beetles or not. For example, one could expect that seeds would be dispersed more often by large dung beetles than by small beetles, because smaller beetles form smaller dung balls and are therefore likely to disperse fewer seeds. Additionally, small seeds are unlikely to disperse seeds that are large relative to their body size.

Several variables can affect the specific composition of the dung beetle assemblage, and may thus indirectly affect the fate of seeds (Fig 2.1). Such variables include: (i) Season of the year (wet vs. dry): several studies in tropical forests have reported higher abundance of dung beetles during the rainy season than during the dry season (Hanski 1980, Janzen 1983, Estrada and Coates-Estrada 1991, Gill 1991, Andresen 1999); (ii) Time of day (day vs. night): dung beetle communities often consist of approximately equal numbers of diurnal and nocturnal species, though in tropical forests the nocturnal guild dominates in terms of biomass (Hanski and Cambefort 1991c); (iii) Amount of dung: A study in a tropical forest in Ecuador showed that dung amount is an important niche dimension along which dung beetles can specialize (Peck and Howden 1984).

The main objective of this study was to ascertain whether dung beetles are secondary seed dispersers in Central Amazonia, and identify some of the factors that affect this plant-animal interaction. Specific objectives of this study were to determine if season, time of day, and dung amount affect the composition of the dung beetle assemblages in Central Amazonia, and to assess if these factors, together with seed size

and beetle size can affect the outcome of the seed-beetle interaction. To address these objectives the following questions were asked:

1. Are there differences in dung beetle assemblages captured:
   a) in dry vs. wet months?
   b) in diurnal vs. nocturnal periods?
   c) with different amounts of dung?

2. How is secondary dispersal of seeds by dung beetles affected by:
   a) seed size?
   b) beetle size?
   c) amount of dung?
   d) time of day?
   e) season?

In summary, this study contributes to the general field of seed dispersal ecology by showing the large effect that secondary dispersers can have on the short-term fate of seeds. This study stresses the importance of considering this stage of the seed dispersal process when one wants to assess the overall effectiveness of the primary dispersal agent, or when one wants to assess the factors that affect plant regeneration. For the more specific field of seed dispersal by frugivorous mammals in tropical forests, this study contributes by increasing the little understanding we have on the role of dung beetles as secondary seed dispersers, and on the factors that affect the outcome of the seed-beetle interaction.

Methods

Study Site

This study was conducted in a 10,000 ha reserve that is part of the Biological Dynamics of Forest Fragments Project (BDFFP): "km 41" (reserve #1501), located *ca.* 90 km north of the city of Manaus (2°30'S, 60°W), in the Brazilian state of Amazonas. The

study site is mainly covered by primary *terra firme* forest (not seasonally flooded). The flora is very rich in tree species and the canopy is approximately 30-37 m tall, with emergent trees reaching 55 m (Lovejoy and Bierregaard 1990). Mean annual temperature in Manaus is 26.7°C and mean annual rainfall is 2,186 mm (Lovejoy and Bierregaard 1990), with a dry season between June and October. Detailed descriptions of the study sites can be found elsewhere (Lovejoy and Bierregaard 1990, Prance 1990, Rankin-de-Merona et al. 1992).

The present study took place between May 1996 and May 1998 (continuous except for two periods of 2 weeks each, and two periods of 1 month each).

The Dung Beetle Community

Dung beetles were sampled using pitfall traps. Traps were plastic containers 15 cm high and 10 cm in diameter. Each trap was filled to approximately two thirds of its capacity with soapy water (odorless soap was used) and buried level with the ground surface. Fresh howler monkey dung was suspended ca. 5 cm above the buried trap, inside a bag made with plastic mosquito netting (see below for bait sizes used). Traps were placed in the forest, under intact canopy, at least 50 m from any treefall gap and with a minimum distance of 30 m between adjacent traps. Traps were located along existing trails, at least 1 m into the forest, away from the trail's edge. The exact location of the trap was determined by the presence of an appropriate vine or branch from which to hang the bait. When heavy rain fell more than two hours of the trapping period, the trapping was cancelled.

Dead dung beetles collected from each trap were put in plastic containers with alcohol. They were later taken to Manaus, where they were sorted by morphospecies.

Samples of each morphospecies were pinned, dried, and measured (body length measured with a caliper to the nearest 10th of a mm). The mean size of beetles was calculated for each trapping period, following Peck and Howden (1984). Mean size of the beetles in a sample is the summation of the number of each species multiplied by its mean length, divided by the total number of beetles caught. Dung beetles were also classified into tow size categories: < 10 mm in length, and $\geq$ 10 mm in length. Beetle size is important because it is directly related to the probability of a beetle moving a seed. In particular, beetles < 10 mm are unlikely to be important in the movement of seeds > 4-5 mm (which is the minimum size used in this study, Feer 1999, Vulinec 1999, Chapter 4).

Dung beetles were also classified according to their dung-processing behavior into "tunnelers", "rollers" and "dwellers". Rollers make a dung ball and always move it away from the source before burying it. In contrast, "tunneler" dung beetles make a burrow close to the dung source and then start provisioning their tunnel, usually making several trips to the dung source (Halffter and Edmonds 1982, Cambefort and Hanski 1991). Finally, the dwellers process the dung immediately below the dung pat, or inside it (Halffter and Edmonds 1982). Only tunneler and roller dung beetles are likely to move seeds horizontaly and/or vertically, and it has been found that tunneler species bury more seeds than roller species, and are consequently thought to be more important in secondary seed dispersal (Estrada et al. 1993, Andresen 1999, Feer 1999).

At the end of the study period the dung beetle collection was donated to the entomological collection of INPA (National Institute for Research in Amazonia), in Manaus. Other specimens were sent for identification to Fernando Z. Vaz-de-Mello

(Setor de Ecologia, Dep. de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36571-000, Brazil).

Because large amounts of fresh fecal material were needed for conducting the beetle trappings as well as the experiments described below, I used the dung of howler monkeys (*Alouatta seniculus*) throughout the study. Howler monkeys are abundant in the study site, and it was relatively easy to locate groups and find large quantities of fresh dung. I usually collected dung in the morning (0700-1000 h). All seeds > 3 mm in the dung were removed to avoid interference with experimental seeds and/or beads in the experiments described below. Seeds ≤ 3 mm (including *Ficus* spp. seeds) were not common in dung, their removal was not practical, and they were considered unlikely to affect the interaction between dung beetles and experimental seeds/beads. The dung was kept in plastic bags in the shade and, since dung freshness is important for its attractiveness to dung beetles (Howden and Nealis 1975, Halffter and Edmonds 1982), it was generally used on the day it was collected. When the dung was used the next day, it was kept in an insulated box with ice.

Effects of season and time of day. To assess the variation in the dung beetle community at different times of the year and of the day, dung beetles were trapped 14 times between August 1996 and January 1998, with at least one month between consecutive trapping periods. Eight of the trapping periods were conducted during months corresponding to the dry season (June-October) and six were conducted during wet months (November-May). During each trapping period 10 diurnal traps and 10 nocturnal traps were set out, each baited with 25 g of dung. The diurnal traps were baited at dawn and dung beetles collected at dusk. Fresh dung was placed at dusk and dung

beetles collected at dawn, for the nocturnal sampling. The ten trap locations were the same for diurnal and nocturnal traps, and were the same in all 14 trapping periods. The exact location of traps was chosen in the same way described above.

Effect of dung amount. To determine if different dung beetle assemblages are attracted to dung piles of different sizes, dung beetles were trapped four times with 5, 10 and 25 g of bait, and twice with 50 g. These sizes were based on the defecation sizes observed in this forest for howler monkeys (Chapter 3). In three of the trapping periods (including those with the 50 g treatment), three traps were used per dung treatment and in the other trapping period two traps were used for each dung treatment. The total number of traps used were 6 traps for the 50 g treatment and 11 traps for each of the other three treatments. Baits of all sizes were used on the same day. In each of the four trapping periods, dung treatments were assigned to different trap locations in the forest. Two of the trapping periods were 24 h in duration. One began in the morning and the other in the afternoon. The other two trapping periods were 16 h in duration, and began in the afternoon. Two of the trapping periods were performed during the dry season of 1996 and two during the wet season of 1998. Due to the differences in seasons, trapping duration, and trap setup time, for statistical analyses I used each of the four trapping periods as a "block" in an incomplete block design (see Data Analysis section below for a more detailed description of statistical analyses).

Secondary Seed Dispersal by Dung Beetles

To quantify the role that the dung beetle community plays in secondarily dispersing seeds present in dung, I performed a series of experiments in which I used seeds of different plant species and/or plastic beads as seed mimics. Seeds are sometimes difficult

to find large quantities in rainforests. Thus, the use of beads is practical when the purpose is to determine the percentage of seeds of different sizes that will potentially be buried by the dung beetle community, as well as the depth to which these will be buried. An additional advantage of using beads is that they are not removed by seed predators (Andresen 1999). Also, by using plastic beads of a standard type one can more easily make regional comparisons regarding the role of dung beetles as seed dispersers. Finally by using plastic beads one can examine the effect of size while keeping constant the shape of the bead (Andresen 1999).

Experiment 1: Effects of seed size and dung amount. In this experiment I used beads of nine different types (beads varied in shape and size, Table 2.1) and three dung amounts to assess the effect of bead size and amount of dung on bead burial by dung beetles. To find beads that were buried and/or moved horizontally by beetles, a 50 cm piece of thin white nylon thread was tied to each bead. Although the thread may interfere with the activity of smaller dung beetles (< 10 mm), making it more likely that small beetles try to "avoid" the tread, these beetles are probably not very important in the movement of seeds/beads > 4-5 mm in length (Figure 2.6). Also, due to differences in dung-relocation behavior, the thread is more likely to interfere with the activity of roller dung beetles, than with the activity of tunneler beetles (see above). And since only 3 roller species were ≥ 10 mm, I believe that in general the presence of the thread did not significantly affect seed movement by dung beetles.

Single beads were put inside dung piles of three sizes: 5 g, 10 g and 25 g. Since dung was limiting during this study, I could not replicate all bead types with all three dung treatments (see Table 2.1 for bead types used with each dung treatment and for

sample sizes). I decided to leave out the heaviest bead from the 5 g treatment because it was likely that very few of those beads would be buried by dung beetles. And I decided to concentrate the 25 g treatment (the most "expensive" treatment in terms of dung) on the larger beads, assuming that large seeds are the ones to benefit more from being surrounded by more dung, in terms of increasing their burial probability. The bead-containing dung piles were placed on the forest floor, along transects, one pile every 10 m. From 10-50 dung piles were put on the forest floor on a single day. All dung piles with beads were set out between 1500 h and 1800 h during the dry season, to avoid having time of day and season as confounding factors. After two days, each bead was recovered (sample sizes for each bead type are given in Table 2.1). I recorded whether it had been buried by dung beetles, the depth of burial to the nearest centimeter (for seeds slightly covered by soil I assigned a burial depth of 0.5 cm), and the horizontal distance moved from the original site to the nearest five centimeters.

Table 2.1. Mass, length and width of beads used as seed mimics to quantify the role of dung beetles as secondary seed dispersers. The sample sizes used in Experiment 1 for the three dung treatments are also given, where N is the number of individual bead-containing dung piles.

Bead Type	Mass (g)	Length (mm)	Width (mm)	N for 5 g	N for 10 g	N for 25 g
# 1	0.03	4.0	3.8	36	44	--
# 3	0.11	5.7	5.5	35	72	--
# 4	0.25	8.0	7.6	36	81	--
# 6	0.23	12.1	5.3	37	40	--
# 8	0.33	18.4	5.5	39	50	47
# 9	0.63	16.0	9.5	27	42	33
# 10	0.87	11.2	10.9	25	41	42
# 11	1.73	25.8	10.9	32	42	44
# 12	2.16	20	13.3	--	34	33

Experiment 2: Effect of dung beetle size. While in the previous experiment I assessed the effect of the entire dung beetle community on secondary dispersal of seeds, in this experiment I tested individual dung beetle species with seeds of different sizes, to assess the importance of dung beetle size on seed burial. To do this, I captured individuals of six dung beetle species (for one species, which was strongly dimorphic in terms of size, I tested small and large individuals separately) and put 1-4 individuals (depending on size) of the same species in a 25 cm diameter plastic bucket, filled with forest soil up to 20 cm. The dung beetle species used in this experiment were: *Scybalocanthon* sp. 1 (length: 10 ± 0.2 mm, N = 10; 4-5 beetles/bucket; here and elsewhere I report means ± one standard error), *Dichotomius lucasi* (12 ± 0.3mm, N = 9; 2-3 beetles/bucket), *Phanaeus chalcomelas* (14 ± 0.3 mm, N = 8; 1-2 beetles/bucket), *Dichotomius* sp.1 (16 ± 0.3 mm, N = 7; 1 beetle/bucket), *Dichotomius subaeneus* (17 ± 0.5 mm, N = 10; 1 beetle/bucket), *Dichotomius boreus* (small individuals: 19 ± 0.9 mm, N = 4; 1 beetle/bucket), *Dichotomius boreus* (large individuals: 25 ± 0.5 mm, N = 10; 1 beetle/bucket). On top of the soil, in the bucket, I deposited a 10 g dung pile with seeds inside and covered the bucket with a lid. I used three different seed species for each species of beetle: *Helicostylis scabra* (length: 5 ± 0.2 mm, N = 10), *Pourouma guianensis* (length: 11 ± 0.8 mm, N = 10) and *Pouteria durlandii* (length: 27 ± 0.7 mm, N = 10). In the dung piles I included seeds of a single species each time, using 8 seeds in the case of *H. scabra*, 4 seeds for *P. guianensis* and 2 seeds for *P. durlandii*. When all the dung had been buried by the beetles (usually within one or two days), I counted the number of seeds remaining on the surface. I repeated the experiment five times per seed species for *D. subaeneus, D.* sp. 1 and *P. chalcomelas*; seven times for *S.* sp. 1, *D. clypeatus* and

small *D. boreus*; and 11 times for large *D. boreus*. Individual dung beetles were re-used up to three times.

Experiment 3: Time of day: day vs. night. The purpose of this experiment was to determine whether a difference exists in seed burial by dung beetles, depending on whether seeds are defecated in the morning, and consequently handled by diurnal dung beetles during the first 10-12 h of deposition, vs. seeds defecated in the late afternoon, and consequently handled mostly by nocturnal dung beetles during the first 10-12 h. I repeated the same setup described in Experiment 1, but placed dung piles in the morning (AM = 0600 – 0700 hrs) and in the late afternoon (PM = 1700 – 1800 hrs). Again, I counted number of seeds buried and measured burial depths after two days. This experiment was conducted for bead #4 (diameter = 8 mm, Table 2.1) with 5 g (N = 33 and 36 dung-bead piles for AM and PM, respectively) and 10 g dung piles (N = 76 and 81 dung-bead piles for AM and PM, respectively), and for *Byrsonima crispa* seeds (diameter = 8 ± 0.2, N = 15) with 10 g (N = 120 and 152 seed-dung piles for AM and PM, respectively) and 25 g dung piles (N = 80 and 120 dung-seed piles for AM and PM, respectively). The PM data for bead #4 were the same gathered in Experiment 1. This experiment was run in the same season and general area as Experiment 1.

To gain a general idea of dung beetle activity during the diurnal and nocturnal periods, I also recorded whether some dung remained 12 hours and 24 hours after setting out the dung piles on the forest floor. Due to time constraints I was not always able to do this monitoring of dung piles. However, I monitored other transects as well, besides the ones set out to assess seed/bead burial at day vs. night, to gather this information. These additional transects were those set out for experiments described elsewhere (Chapters 4

and 5). This allowed me to complete large sample sizes, which are shown in Fig. 2.9. Data were gathered only during dry seasons.

Experiment 4: Season of the year: dry vs. wet. To assess the effect of season on seed dispersal by dung beetles I performed five paired comparisons of seed/bead burial in the wet and in the dry season. Only for one paired comparison did I have data available for the same seed species in both seasons; for the other four comparisons, I paired different seed species, or seeds and beads with similar sizes and shapes. For the paired comparisons I used bead burial data from Experiment 1, and seed burial data from Chapter 4. The five paired comparisons were as follows:

a) *Pourouma guianensis* seeds (length: 11 ± 0.8 mm, width: 7 ± 0.3 mm, thickness: 6 ± 0.3 mm, N = 10) in the wet season paired with *Ocotea percurrens* seeds (length: 12 ± 0.2 mm, width: 8 ± 0.1 mm, thickness: 7 ± 0.1 mm, N = 10) in the dry season.

b) *Micropholis guyanensis* seeds (length: 18 ± 0.3 mm, width: 9 ± 0.2 mm, thickness: 6 ± 0.2 mm, N = 20) compared in both seasons.

c) *Buchenavia grandis*, small seeds (length: 21 ± 0.3 mm, width: 10 ± 0.1 mm, thickness: 10 ± 0.1 mm, N = 10) in the wet season paired with *Minquartia guianensis* seeds (length: 22 ± 0.5 mm, width: 12 ± 0.6 mm, thickness: 12 ± 0.6 mm, N = 10) in the dry season.

d) *Buchenavia grandis*, large seeds (length: 30 ± 0.4 mm, width: 13 ± 0.3 mm, thickness: 12 ± 0.3 mm, N = 10) in the wet season paired with bead # 11 (length: 26 mm, width: 11 mm, thickness: 11 mm) in the dry season, using 10g dung piles.

e) Same as d) but with 25 g dung piles.

As in the previous experiment, I also gathered information regarding the general activity of dung beetles in dry vs. wet season, by assessing rates of dung removal after 24 hours in both seasons, for 10 g dung piles placed in the afternoon. For the dry season I used data for the 10 g dung piles placed in the afternoon in Experiment 3 (N = 179), and for the wet season I monitored 298 additional 10 g dung piles during the rainy months.

Data Analysis

The dung beetle community. For analyses, for each diurnal and nocturnal trapping period (14 of each), the total number of dung beetle individuals and the total number of species captured were added over the ten traps used in each period. Thus for calculating means, each period was treated as a sample. Three, two-way analyses of variance (ANOVA) were carried out to determine the effect of season (dry vs. wet), the effect of time of day (day vs. night), and their interaction, on number of beetles captured, number of species captured and mean beetle size. Data for number of individuals and number of species were square-root-transformed and data for mean beetle size were log-transformed. Additionally, the total number of beetles < 10 mm and beetles ≥ 10 mm for all day trapping periods together and all night trapping periods together were calculated and analyzed with a Pearson Chi-squared test.

To analyze the effects of the amount of dung, the average number of individuals and species per trap was used rather than the total number, because the number of traps used was not equal in all trapping periods (see above). And, because trapping periods were not homogeneous in terms of number of hours of sampling and time of day at which sampling began, each trapping date was used as a block in an incomplete block design ANOVA. This analysis was used to assess the effect of dung amount on the number of beetles captured (square-root-transformed), number of species captured, and mean size of beetles. The Tukey test for adjusted post-hoc pairwise comparisons was used to compare pairs of dung treatments. Also, the test of linear contrasts (SPSS Inc. 1996) across ordered groups of means was used to test whether the number of beetles and species captured increased linearly with increasing amount of dung. The total frequency of

beetles < 10 mm and ≥ 10 mm captured with different amounts of dung were analyzed with a Pearson Chi-squared test.

Secondary seed dispersal by dung beetles. Bead mass is highly correlated with bead size (both length and width), and, since the beads used had different shapes, I chose to use bead mass as a better indicator of overall bead size. To analyze the effects of bead mass and amount of dung surrounding the bead on percentage of beads buried and depth of burial, I first carried out two linear regressions for each dung treatment (5 g, 10 g and 25 g). In these regressions the independent variable was bead mass, and the dependent variables were percentage of beads buried and depth of burial. Then I tested for homogeneity of slopes among the three dung treatments for both independent variables. Finally I performed two analyses of covariance (ANCOVA), to test for differences in percentage of beads buried and depth of burial among the dung treatments, using bead mass as the covariate.

To analyze the effect of bead mass and of dung amount on percentage of beads moved horizontally by dung beetles and on the distance moved, I carried out linear regressions for each dung treatment. Again, the independent variable was bead mass, and the dependent variables were percentage of beads moved and distance moved. Since only the regressions for 10 g of dung were found to be significant, ANCOVA's were not performed. Differences among dung treatments for the percentage of beads moved horizontally were tested by analyzing frequencies with a Pearson Chi-square test and Cochran's test for linear trend, which is a test designed to reveal whether proportions increase (or decrease) linearly across ordered categories (SPSS Inc. 1996). Differences

among dung treatments for the horizontal distances were tested with a one-way ANOVA (log-transformed data).

To test for the effect of dung beetle size on percentage of seeds buried, linear regressions were performed for the three seed species used, with beetle size as the independent variable and percentage of seeds buried (transformed as the arcsine of proportion of seeds buried) as the dependent variable. Since the slopes of the three regressions were not homogeneous, an ANCOVA could not be performed to test for differences in percentage of seeds buried among the three seed species (Sokal and Rohlf 1995). Instead, frequencies were analyzed with Pearson Chi-square tests and the Cochran test for linear trend.

To assess the effect of time of day in which seeds/beads were placed on the forest floor on percentage of seeds/beads buried, data for bead # 4 were analyzed with Fisher exact tests, using total frequencies. For *B. crispa* seeds, samples were larger, allowing seeds to be pooled in groups of 8 seeds to be analyzed with t-tests (I chose to pool the seeds in groups of 8, because it is a small number that still allows me to obtain a normally distributed variable, expressed as the percentage of the 8 seeds that were buried by beetles; I pooled seeds that were adjacent in a transect). Additionally a two-way ANOVA was performed for *B. crispa* seeds to test for the interaction between time of day and dung amount. To test the effect on burial depth, Mann-Whitney U tests and t-tests were used (square-root-transformed data). For *B. crispa* seeds, again a two-way ANOVA was done to test for interaction between time of day and dung amount.

To analyze the effect of the season in which dung with seeds/beads was placed on the forest floor on the number of seeds buried and burial depth, Pearson Chi-square tests

and t-tests were used, respectively, for each of the five independent bead/seed pairs. Additionally, Wilcoxon signed ranks test was used to test the overall effect of season, using each seed/bead pair as an independent sample.

Pearson Chi-square tests were used to analyze the data on dung removal after 12 and 24 h for dung piles placed in the afternoon vs. dung piles placed in the morning, and also for dung removal after 24 h for dung piles placed in the wet vs. dry season.

All tests were two-tailed. The level of significance was 0.05. When performing post-hoc pairwise comparisons the level of significance was adjusted for individual tests, in order to maintain the overall significance level of 0.05 (e.g., the Tukey adjusted comparisons after the incomplete block design ANOVA).

All analyses were performed using Systat® 6.0 (SPSS Inc. 1996).

Results

The Dung Beetle Community

Effects of season and time of day. A total of 8,845 dung beetles were captured in the 14 diurnal and 14 nocturnal trapping periods. Sixty-one species of dung beetles were captured; 59 belonged to the family Scarabaeidae and 2 to the family Aphodiidae. Twenty-six species were mainly caught in the nocturnal traps, 31 mainly in the diurnal traps, and four species were equally frequent in both types of traps. Thirty-two species were tunnelers, 10 were rollers, 2 were dwellers, 3 species of the genus *Eurysternus* show a very particular type of dung processing behavior in which the dung balls are only shallowly covered by soil (Halffter and Edmonds 1982), and 14 unidentified species were not classified according to their dung-processing behavior (only two of these were

≥ 10mm in length). Dung beetle species showed a wide range of body sizes, from 2 mm to 40 mm. Thirty percent of the species (11 tunneler species, 3 roller species, 2 *Eurysternus* species, and 2 unidentified species) and 16% of all individuals captured were ≥ 10mm in length (Appendix A).

The average number of beetles captured in diurnal trapping periods (252 individuals/10 traps) was significantly lower than the number of beetles captured in nocturnal trapping periods (380 individuals/10 traps). Although the average number of beetles captured was higher in the wet season than in the dry season (for both diurnal and nocturnal trapping periods), there was neither a statistically significant effect of season nor a significant interaction between season and time of day (two-way ANOVA, time of day: $F_{1,24} = 5.93$, $P = 0.02$; season: $F_{1,24} = 2.21$, $P = 0.15$; time of day x season: $F_{1,24} = 0.01$, $P = 0.92$; Fig. 2.2 a). Great variability was observed in monthly captures of dung beetles as well as in rainfall (Fig. 2.3)

The mean number of beetle species captured in the dry season (37 species/10 traps) was significantly lower than the number of beetle species captured in the wet season (42 species/10 traps), while time of day and the interaction term had non-significant effects (two-way ANOVA, time of day: $F_{1,24} = 2.63$, $P = 0.12$; season: $F_{1,24} = 6.30$, $P = 0.02$; time of day x season: $F_{1,24} = 0.19$, $P = 0.67$; Fig. 2.2 b).

The mean length of beetles was significantly smaller for beetles captured in diurnal trapping periods (5.3 mm) than for beetles captured in nocturnal trapping periods (6.9 mm). There was no effect of season or the interaction term on the mean size of beetles (two-way ANOVA, time of day: $F_{1,24} = 22.37$, $P < 0.01$; season: $F_{1,24} = 0.27$, $P = 0.61$; time of day x season: $F_{1,24} = 0.55$, $P = 0.46$; Fig. 2.2 c). When *Uroxys* sp., a very small

species that can be extremely abundant in nocturnal traps is excluded, the mean size of beetles captured in nocturnal trapping periods increases to 8.7 mm. Only seven percent of beetles captured during diurnal trapping periods were ≥ 10 mm in length, while in the nocturnal trapping periods 21% of beetles were in this size category ($\chi^2 = 329.15$, d.f. = 1, P < 0.01).

Effect of dung amount. To assess the differences in the numbers of species and individuals attracted to different amounts of dung, beetles were captured using four different bait sizes: 5 g, 10 g, 25 g and 50 g of dung. There were significant differences among the four dung treatments in mean number of individuals captured per trap (incomplete block design ANOVA, $F_{3,7} = 12.11$, P < 0.01) and mean number of species per trap (incomplete block design ANOVA, $F_{3,7} = 14.78$, P < 0.01). For both variables there was a significant linear increase with increasing amounts of dung (tests of linear contrast, number of individuals: $F_{1,7} = 33.44$, P < 0.01; number of species: $F_{1,7} = 41.22$, P < 0.01). Also, for both variables, no significant differences were found between 5 g and 10 g (individuals: P = 0.26, species: P = 0.10), between 10 g and 25 g (individuals: P = 0.18, species: P = 0.32), and between 25 g and 50 g (individuals: P = 0.28, species: P = 0.14), when analyzed with the Tukey adjusted post-hoc pairwise tests. However, there were statistically significant differences between 5 g and 25 g (individuals: P = 0.01, species: P = 0.01), 5 g and 50 g (individuals: P < 0.01, species: P < 0.01), and 10 g and 50 g (individuals: P = 0.03, species: P = 0.02; Fig. 2.4).

No differences were found among dung treatments in terms of mean size of beetles, which was 5 mm for the 5 g and 50 g treatments, and 6 mm for the 10 g and 25 g treatments (incomplete block design ANOVA, $F_{3,7} = 0.68$, P = 0.59). Eighty five percent
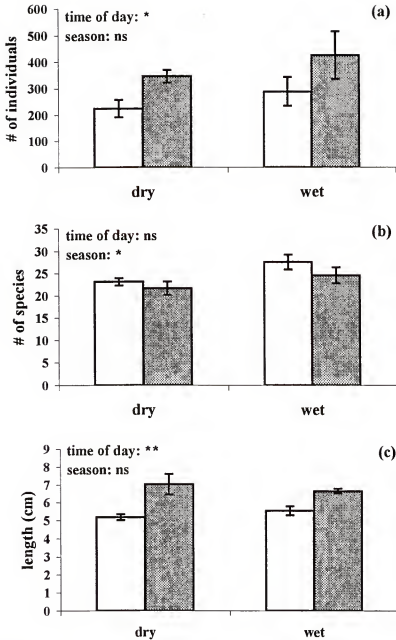
Figure 2.2 Average number of dung beetles (a), average number of dung beetle species (b), and mean beetle size (c), for beetles captured in ten pitfall traps at different times of day (day [white bars] and night [grey bars]) and at different seasons (dry and wet). Bars indicate ± 1 standard error. For the statistical significance of the effects of time of day and season, ns is for P > 0.05, * is for P < 0.05, and ** for P < 0.01.
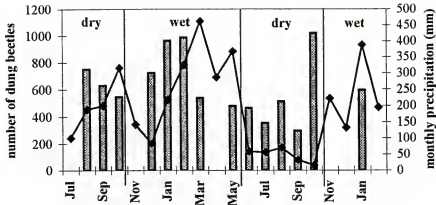
Figure 2.3 Monthly rainfall (line) and mean number of beetles captured/10 traps (bars). Months with no bars mean that no sampling of beetles was conducted during that month.
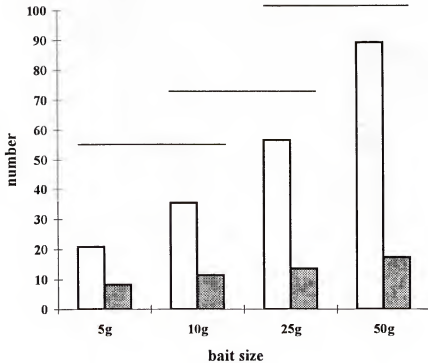
Figure 2.4 Mean number of individuals (white bars) and number of species (grey bars) captured per trap, using as bait four different amounts of dung. The horizontal lines above the bars bind together treatments that are not statistically different.

of all beetles captured with both 5 g and 10 g baits, were < 10 mm in length, while 87% of the beetles captured with both 25 g and 50 g baits were < 10 mm in length ($\chi^2 = 0.69$, d.f. = 3, P = 0.88).

Secondary Seed Dispersal by Dung Beetles

Experiment 1: Effects of seed size and dung amount. Nine different types of beads were used as seed mimics and placed inside dung piles of 5 g, 10 g, and 25 g. Between 6% and 73% of these beads were buried by dung beetles, depending on both bead mass and amount of dung. For all three treatments of dung amount a significant negative linear relationship existed between percentage of beads buried and bead mass (linear regressions, 5 g: %burial = 45.87 − 24.28*bead mass, $R^2 = 0.79$, $F_{1,6} = 22.14$, P < 0.01; 10 g: %burial = 54.81 − 22.98*bead mass, $R^2 = 0.78$, $F_{1,7} = 25.21$, P < 0.01; 25 g: %burial = 62.89 − 15.47*bead mass, $R^2 = 0.96$, $F_{1,3} = 73.50$, P < 0.01). The slopes of the three regression lines were homogeneous ($F_{2,16} = 0.87$, P = 0.44) and the subsequent ANCOVA (with bead mass as the covariate) yielded significant differences in percentage of bead burial among the three dung treatments, with proportionately more beads being buried with increasing amounts of dung ($F_{2,18} = 13.49$, P < 0.01; Fig. 2.5 a).

Mean burial depths varied between 0.5 and 7 cm; the maximum burial depth was 35 cm. For the 10 g treatment a significant linear relationship existed between burial depth and bead mass, with heavier beads being buried less deeply (linear regression, depth = 4.32 − 1.48*bead mass, $R^2 = 0.62$, $F_{1,7} = 14.99$, P = 0.01). For the 5 g treatment a significant linear relationship was found after removing one outlier (linear regression with outlier removed, depth = 2.40 − 1.22*bead mass, $R^2 = 0.80$, $F_{1,5} = 20.25$, P = 0.01). And for the 25 g treatment the linear relationship was suggestive of significance (linear
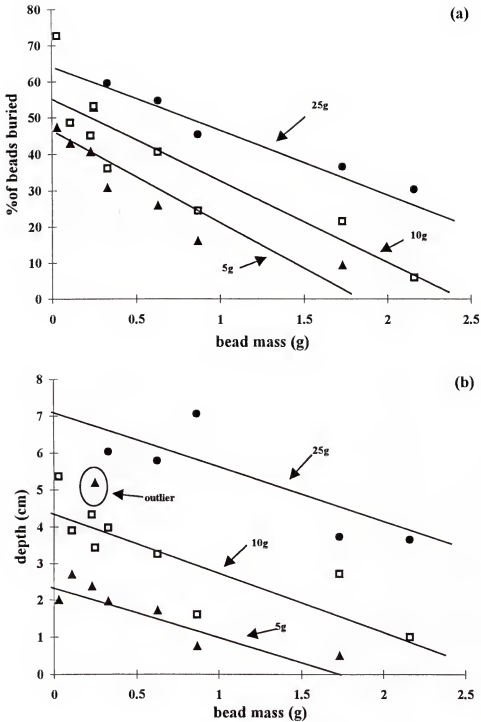
Figure 2.5 Relationship of bead mass with the percentage of beads buried by dung beetles (a), and with the depth of burial (b). Beads were surrounded by three different amounts of dung: 5 g (triangles), 10 g (squares), and 25 g (circles). Also indicated in (b) is the outlier that was removed for the linear regression analysis (see text).
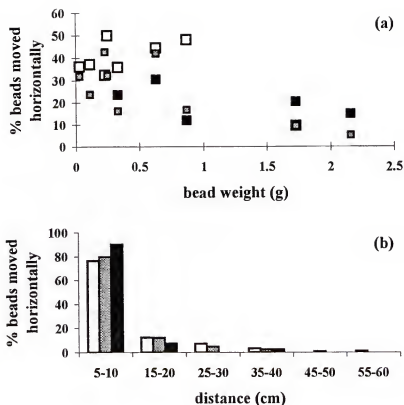
Figure 2.6 Percentage of beads of different sizes moved horizontally by dung beetles (a), and horizontal distance moved (b), for beads with 5g (white squares and bars), 10 g (grey squares and bars), and 25 g of dung (black squares and bars). Horizontal distances were measured to the nearest 5 cm. Sample sizes for (a) are given in Table 2.1, sample sizes for (b) are 97, 123 and 40 beads for the 5 g, 10 g and 25 g dung treatments, respectively.

regression, depth = $7.13 - 1.65$*bead mass, $R^2 = 0.93$, $F_{1,3} = 7.42$, $P = 0.07$). The slopes of the three lines were found to be homogeneous ($F_{2,15} = 0.19$, $P = 0.83$) and the ANCOVA yielded significant differences in burial depth among the three dung treatments, with beads buried more deeply when surrounded by larger amounts of dung ($F_{2,17} = 52.06$, $P < 0.01$; Fig. 2.5 b).

Beads that were not buried by dung beetles remained on the soil surface but were clean of dung. Between 5% and 44% of beads were moved horizontally by dung beetles (including beads buried by beetles and those remaining on the surface). Mean distances varied between 6 and 17 cm, with a maximum distance of 60 cm (Fig. 2.6). Only in the 10 g treatment did both percentage of beads moved and horizontal distance display a significant linear relationship with bead mass, negative for the former and positive for the latter (linear regressions, % seeds moved = $33.50 - 13.04$*mass, $R^2 = 0.53$, $F_{1,7} = 7.77$, $P = 0.03$; log [horizontal distance] = $2.07 + 0.48$*mass, $R^2 = 0.66$, $F_{1,7} = 13.46$, $P = 0.01$). None of the relationships with 5 g and 25 g of dung was significant (all P-values > 0.1).

The total percentage of seeds moved horizontally decreased linearly with increasing amount of dung. With 5 g of dung, 36% of seeds were moved, with 10 g of dung 25% were moved and with 25 g of dung 20% were moved ($\chi^2 = 17.79$, d.f. = 2, $P < 0.01$; Cochran's statistic of linear trend = 16.51, d.f. = 1, $P < 0.01$). When the three bead types with the smaller masses (those that were not tested with the 25 g treatment) were excluded from the analyses, the differences and the linear trend were still significant.

Finally, the mean distances to which the beads were moved horizontally did not differ among the three dung-amount treatments (one-way ANOVA, $F_{1,19} = 2.17$, $P = 0.14$).
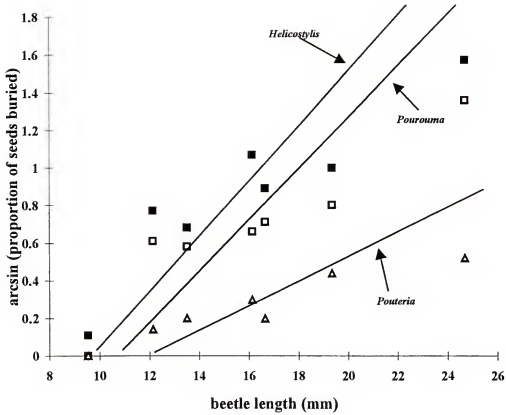
Figure 2.7 Relationship between proportion of seeds buried and dung beetle size, for three species of seeds: *Helicostylis scabra* (length: 5 mm, closed squares), *Pourouma guianenesis* (length: 11 mm, open squares), and *Pouteria durlandii* (length: 27 mm, triangles).

Experiment 2: Effect of dung beetle size. To test the effect of beetle size on seed burial, I placed several individuals of specific dung beetle species of seven sizes in buckets and presented them with dung piles containing seeds of three plant species. For each of the seed species, a significant positive linear relationship existed between percentage of seeds buried and beetle size (linear regressions, *Helicostylis scabra* seeds: arcsine [proportion of seeds buried] = - 0.45 + 0.08*beetle length, R^2 = 0.87, $F_{1,5}$ = 32.25, P < 0.01; *Pourouma guianensis* seeds: arcsine [proportion of seeds buried] = - 0.52 + 0.07*beetle length, R^2 = 0.88, $F_{1,5}$ = 36.70, P < 0.01; *Pouteria durlandii* seeds: arcsine [proportion of seeds buried] = - 0.29 + 0.29*beetle length, R^2 = 0.92, $F_{1,5}$ = 53.85, P < 0.01). The slopes of the three regression lines were not homogeneous ($F_{2,15}$ = 5.20, P < 0.02) and thus an ANCOVA could not be carried out to detect differences among the three seed species. Analyzing the total frequency of seed burial for each species, pooling the data for all beetles, 73% of *H. scabra* seeds were buried, 64% of *P. guianensis* seeds were buried, and 28% of *P. durlandii* seeds were buried. The percentage of seeds buried significantly decreased with increasing seed size (χ^2 = 66.52, d.f. = 2, P < 0.01; Cochran's test for linear trend = 57.36, d.f. = 1, P < 0.01; Fig. 2.7).

Experiment 3: Time of day: day vs. night. I compared percentage of seeds buried and burial depth for *B. crispa* seeds and bead # 4 placed in the late afternoon (i.e., seeds mostly handled by nocturnal beetles during the first 10-12 h of deposition), with seeds and beads placed in the early morning (i.e., seeds mostly handled by diurnal dung beetles during the first 10-12 h). For *B. crispa* the experiment was done with 10 g and 25 g of dung and for bead # 4 it was done with 5 g and 10 g of dung.
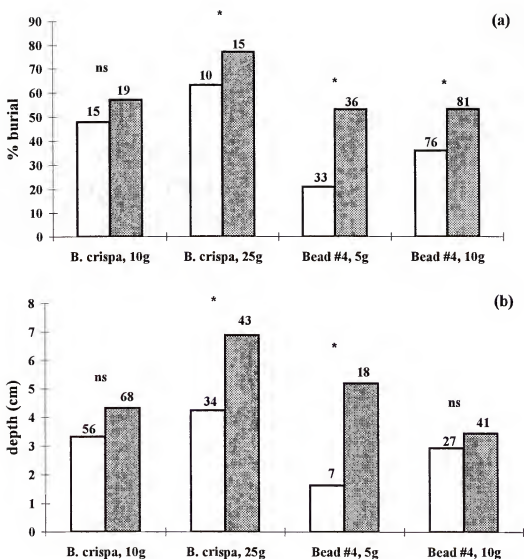
Figure 2.8 Percentage of seed/bead burial (a), and burial depth (b) for seeds/beads placed on the forest floor in the morning (white bars) and in the late afternoon (grey bars). Seeds of *Byrsonima crispa* were used with 10 g and 25 g of dung, and bead #4 was used with 5 g and 10 g of dung. * indicates a statistically significant difference (P ≤ 0.05), and n.s. indicates no significant difference (P > 0.05). Numbers inside the bars are sample sizes, which in the case of (a) for *B. crispa* is the number of groups of 8 pooled seeds, in all other cases the sample size is the number of individual seeds/beads.

For all four comparisons, more seeds were buried when placed in the afternoon than when placed in the morning (Fig. 2.8 a). For bead # 4, 36% of the beads with 10 g of dung were buried when placed in the morning, while 53% were buried when placed in the afternoon (Fisher exact test: P = 0.04). Similarly, for beads with 5 g of dung, 21% were buried when placed in the morning, while again 53% were buried when placed in the afternoon (Fisher exact test: P = 0.01). For *B. crispa* seeds with 10g of dung 48% and 57% of seeds were buried when placed in the morning and in the afternoon respectively, but this difference was not significant (t-test, T = -1.21, d.f. = 32, P = 0.23). For seeds with 25 g of dung 63% and 77% of seeds were buried when placed in the morning and in the afternoon respectively (t-test, T = -2.17, d.f. = 23, P = 0.04). A two-way ANOVA for *B. crispa* revealed significant effects for time of day and for dung treatments, but no significant interaction between them (time of day: $F_{1,55} = 5.04$, P = 0.03; amount of dung: $F_{1,55} = 10.68$, P < 0.01; time of day x amount of dung: $F_{1,55} = 0.26$, P = 0.61).

For all four comparisons, seeds were buried more deeply when placed in the afternoon than when placed in the morning (Fig. 2.8 b). The differences were significant for *B. crispa* seeds with 25 g of dung (t-test, T = -2.29, d.f. = 75, P = 0.03) and for bead # 4 with 5 g of dung (Mann-Whitney U test, U = 23.50, N = 7 for morning and 18 for afternoon piles, P = 0.02). The other two comparisons were not significant (P's > 0.05). Again, a two-way ANOVA for *B. crispa* yielded significant effects for time of day and for dung treatments, but no significant interaction between them (time of day: $F_{1,172} = 6.08$, P = 0.01; amount of dung: $F_{1,172} = 6.91$, P = 0.01; time of day x amount of dung: $F_{1,172} = 1.97$, P = 0.16).
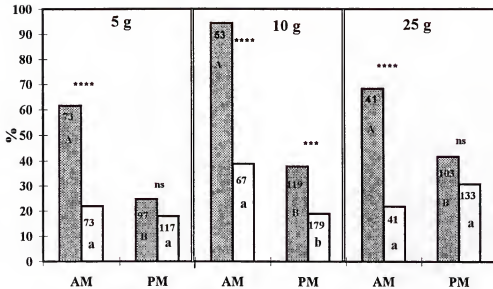
Figure 2.9 Percentage of 5 g, 10 g and 25 g dung piles that still had some dung remaining after 12 hours (grey bars), and after 24 hours (white bars). Dung piles were either placed in the early morning (AM) or in the late afternoon (PM). Statistical analyses were performed for each dung treatment separately. Statistical results for the comparisons between dung remaining after 12 h and after 24 h are given above each pair of bars, where ns is for $P > 0.05$, *** is for $P < 0.001$ and **** is for $P < 0.0001$. Statistical result for the comparisons between AM and PM samples within each time interval are indicated by letters inside the bars: capital letters for the comparisons in the 12 h period, and small-case letters for the comparisons in the 24 h period; different letters (A and B, or a and b) indicate significant differences with P's < 0.005, and equal letters (a and a) indicate non-significant differences with $P > 0.05$. Numbers inside each bar indicate the number of individual dung piles monitored.

In terms of dung removal, more dung remained on the surface after the diurnal period of dung beetle activity (AM) than after the nocturnal period (PM; Fig. 2.9). After 12 hours, significantly more dung remained on the surface in AM dung piles than in PM piles, in all dung treatments (5 g: $\chi^2 = 23.52$, d.f. = 1, P < 0.0001; 10 g: $\chi^2 = 47.38$, d.f. = 1, P < 0.0001; 25 g: $\chi^2 = 8.27$, d.f. = 1, P = 0.004). However, after 24 h, i.e. after all dung piles had passed through a nocturnal period, the difference in dung removal between AM and PM dung piles was only significant in the 10 g treatment ($\chi^2 = 10.37$, d.f. = 1, P = 0.0013) but not in the other two (5g: $\chi^2 = 0.45$, d.f. = 1, P = 0.5; 25 g: $\chi^2 = 1.21$, d.f. = 1, P = 0.27).

Experiment 4: Season of the year: dry vs. wet. I performed five paired comparisons of seed/bead burial in the wet and in the dry season. Each pair consisted of seed species or seeds and beads of similar size and shape. One member of each pair was used during the dry months and the other during the wet months. For all five comparisons the percentage of seeds/beads buried was higher during the wet season. A test of all data considered together, taking each paired comparison as an independent sample, revealed significantly higher bead burial during the rainy season than during the dry season (Wilcoxon signed ranks test, $T^+ = 15$, N = 5, P = 0.03). When each pair was examined individually for significance, the difference between the dry and the rainy season was only significant in two of the pairs: *Pourouma* vs. *Ocotea* ($\chi^2 = 4.26$, d.f. = 1, P = 0.04), and *Micropholis* vs. *Micropholis* ($\chi^2 = 3.92$, d.f. = 1, P = 0.05; Fig. 2.10)

Regarding the depth of burial, data were only available for four of the five pairs. Of these, the burial depth was lower during the rainy season for 3 of the pairs (*Pourouma* vs. *Ocotea*: 4.7 cm vs. 5.3 cm; large *Buchenavia* vs. bead#11 with 10 g of dung: 2.2 cm vs.
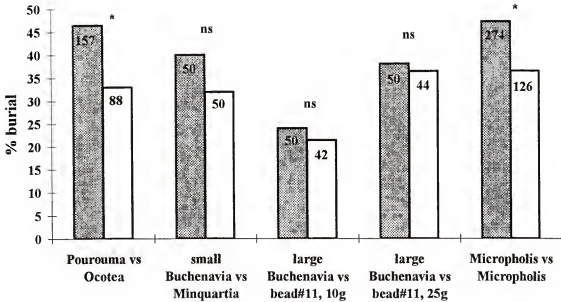
Figure 2.10 Percentage of seed/bead burial in the wet (grey bars) vs. dry (white bars) seasons. Each paired comparison consists of seed species pairs or seed/bead pairs that are similar in size and shape. Numbers inside the bars are sample sizes.

Table 2.2 Summary of objectives, methods and results.

1. THE DUNG BEETLE COMMUNITY : *pitfall traps with fresh howler monkey dung as bait*

a) Effect of season

all baits: dry season { night / day }
* more individuals captured at night
* more species captured during the wet season
* larger beetles captured at night

25 g of dung   wet season { night / day }

b) Effect of time of day
* no effect of season on number of individuals captured
* no effect of time of day on number of species captured
* no effect of season on mean size of beetles captured

c) Effect of dung amount — baits: 5 g, 10 g, 25 g, 50 g
* more individuals and more species captured with larger baits
* no effect of bait size on mean size of beetles captured

2. DUNG BEETLES AS SECONDARY SEED DISPERSERS : *plastic beads and/or seeds are placed inside dung piles and their fate recorded*

a) Effect of seed size

5 g dung piles: 8 bead sizes
* small beads are buried more often than large beads

10 g dung piles: 9 bead sizes
* small beads are buried more deeply than large beads

b) Effect of dung amount

25 g dung piles: 5 bead sizes
* seeds with more dung are buried more often than seeds with less dung
* seeds with more dung are buried more deeply than seeds with less dung

c) Effect of beetle size   beetles about 7 sizes, each tested with 3 seed species of different sizes.
* larger beetles bury a larger percentage of seeds than smaller beetles
* as with beads, smaller seeds were buried less often than larger seeds

d) Effect of time of day

seed/bead burial: day / night } 4 paired comparisons
* seeds/beads are buried more often when set out in the evening
* seeds/bead were buried more deeply when set out in the evening

dung removal: day / night } monitored after 12 h and 24 h
* dung is removed more quickly when set out in the evening

e) Effect of season

seed/bead burial: dry / wet } 5 paired comparisons
* seeds/beads are buried more often when set out during the wet season
* season had no effect on burial depth

dung removal: dry / wet } monitored after 24 h
* dung is removed more quickly when set out during the wet season

2.7 cm; large *Buchenavia* vs. bead#11 with 25 g of dung: 2.8 vs. 3.7 cm) and higher during the rainy season for one of the pairs (*Micropholis* vs. *Micropholis*: 5.2 cm vs. 4.2 cm). However, none of these differences were statistically significant (for all t-tests, P > 0.1).

Finally, in terms of dung removal in the wet vs. dry season, significantly more dung piles had some dung remaining on the surface after 24 h, during the dry months (18.99%), than during the wet months (8.72%; χ^2 = 10.73, d.f. = 1, P = 0.001).

Discussion

The dung beetle community in my study site in Central Amazonia was rich in species (61) as well as in number of individuals (120-706 individuals/10 traps in 12 hours with 25 g bait). Dung beetles buried between 6% and 73% of beads surrounded by dung. This means that the forest in Central Amazonia holds a dung beetle community that is probably playing an active role in the seed dispersal of plant species dispersed through mammalian defecation. Consequently, studies addressing the seed dispersal ecology of such seeds needs to take the activity of dung beetles into consideration.

Time of day, season, and the amount of dung used as bait, significantly affected the composition of the dung beetle assemblage captured in pitfall traps. Time of day and amount of dung had an effect on the number of individuals captured, with more dung beetles captured at night and with larger baits. Time of day also had an effect on mean beetle size, with larger beetles being captured at night, while amount of dung also had an effect on the number of species, with more species being captured with more dung. Season, on the other hand, had only an effect on the number of dung beetle species

captured, with more species being captured during the rainy season. From the perspective of secondary dispersal, it is likely that number of individuals and mean size of beetles attracted to dung are more important than the number of species. Thus, time of day and amount of dung are probably the factors that have a larger influence on secondary seed dispersal by dung beetles, for seeds of a given size.

Time of day and dung amount affected the secondary dispersal of seeds. In particular, seeds were buried more often and more deeply when placed at night and when surrounded by more dung. Season of the year also had an effect on secondary seed dispersal: more seeds were buried during the wet season. As discussed below, the effect of season on seed burial may be caused by other factors related to seed burial and affected by season. Finally, I found that seed size and beetle size are also very important factors in determining how many seeds are secondarily dispersed by dung beetles through seed burial.

Table 2.2 provides a brief summary of the objectives of this study, the methods applied to assess them, and the results found for each. In the following sections, I will first focus on the dung beetle community and the factors determining the composition of the dung beetle assemblage. Then I will discuss how these factors, together with seed size and beetle size, affect the outcome of the seed-dung beetle interaction.

The Dung Beetle Community

Time of day. The nocturnal dung beetle assemblage consisted of approximately 20% more individuals than the diurnal assemblage. Although the species captured at night were mostly different from those captured during the day (Appendix A), the number of species was similar in both periods. Dung beetles captured in the nocturnal

trapping periods were on average larger than beetles captured during the day. Also, the proportion of dung beetles that were ≥ 10 mm in length was three times higher in nocturnal samples (21% of all individuals) than in diurnal samples (7%).These results coincide with what has been found in other tropical forest dung beetle communities in terms of biomass and number of species, although higher number of individuals has not been reported for diurnal samples, compared to nocturnal samples (Hanski and Cambefort 1991c, and references therein).

The differences in the diurnal and nocturnal assemblages translated into differences at the level of secondary seed dispersal (see below).

Season. Dung beetle captures in the rainy season had a significantly higher number of species (42) than samples in the dry season (37). The number of individuals captured was 11% higher during the rainy season than during the dry season. However, this difference was not statistically significant, and there was no difference in the size of beetles in dry vs. wet seasons.

Great variability existed in the number of beetles captured among the 10 traps within trapping periods, as well as among trapping periods within seasons. Rainfall patterns also showed great variability among months within season and among years (Fig. 2.3), and could partly explain the variability in dung beetle abundance. During the study period the average monthly rainfall in wet months (November-May) was 257 ± 36 mm (N = 11), and 115 ± 33 (N = 9) in dry months (June-October).

In other Neotropical forest sites with not very pronounced seasons, researchers have also found little difference in dung beetle assemblages among months (Peck and Forsyth 1982, Waage and Best 1985). In forests where pronounced seasonality in dung beetle

communities has been reported, dung beetles are much more abundant during the rainy season than during the dry season (Hanski 1980, Janzen 1983, Estrada and Coates-Estrada 1991, Gill 1991, Andresen 1999). It is likely that in such forests secondary dispersal by dung beetles will be less important or even absent during the dry months. Low beetle abundance during the dry season is thought to be caused by factors such as increased soil hardness, low soil humidity, and possibly even low fruit availability, since dung beetles often feed on rotting fruit (Janzen 1983, Estrada et al. 1993). In forests with moderate to pronounced seasons most tree species with animal-dispersed seeds produce fruits during the rainy season (e.g., Foster 1984, Terborgh 1986a), which would coincide with the time of high beetle abundance. Seeds that are dispersed through defecation during the dry season, however, are likely to suffer increased levels of seed predation (Janzen 1983, 1986) or fungal attack (Jones 1994) due to reduced seed burial and dung removal rates.. If this is so, it is possible that seeds dispersed during this season have alternative means of lowering predation rates, such as chemical or physical defenses, compared to seeds dispersed during the rainy season.

Amount of dung. Both the number of individuals and the number of dung beetle species captured per trap increased with increasing bait size. The number of individuals captured increased by 62% when comparing baits of 50 g and of 5 g of dung, while the number of species increased by 36%. The greater number of species captured with more dung may just be a consequence of the greater number of individuals captured (sampling effect), while this, in turn, is likely due to larger baits taking more time to dry out and lose their "attractiveness", than smaller baits.

No differences were found in the mean size of the beetles attracted to baits of different sizes. This means that both small and large amounts of dung attract large beetles, which are the most important in secondary seed dispersal (at least for seeds of the size considered in this study). This result was not expected. It is known that larger beetles require and use larger amounts of dung (Doube 1990, Hanski and Cambefort 1991a,c) and thus I expected that larger baits would attract a higher proportion of large beetles than would smaller baits. Researchers in a Panamanian rainforest found that the mean size of dung beetles caught with large baits was nearly twice that of those caught with small baits (Peck and Howden 1984). In that study the size difference between the two bait sizes used was one hundred fold (2 ml vs. 200 ml), while in the present study the difference between the largest and the smallest bait was only tenfold (5 g – 50 g, representing the most common range of sizes of individual fecal clumps found in howler monkey defecation samples, see Chap. 3). A tenfold difference in bait size may not be enough to detect or cause differences in the size distribution of beetles attracted.

Secondary Seed Dispersal by Dung Beetles

Time of day, beetle size and seed size. Larger dung beetles bury more seeds than small beetles; beetle size was found to explain 87-92% of the variation in percentage of seeds buried for three seed species. Larger beetles bury more seeds primarily because they bury larger amounts of dung for feeding and/or oviposition (Doube 1990, Hanski and Cambefort 1991c). Thus, since large beetles bury more seeds and large beetles are more abundant during the night, it was not surprising to find that in the day vs. night comparisons, seeds and beads were buried more often at night than during the day, although one of the comparisons was not statistically significant. Also, dung beetles were

more abundant at night, which also is likely to increase the probability of a seed being buried.

Dung does not always disappear in a single night or a day. Consequently, seeds present in mammal defecations will often be subject to the activity of both diurnal and nocturnal dung beetles. In Experiment 3, beads and seeds were left on the forest floor for two days before checking for burial and burial depth. Still the differences were significant between seeds placed in the morning vs. afternoon, suggesting that the first hours of dung beetle activity after dung deposition are probably most important in determining whether a seed is buried. This is probably because most dung beetle species exploit fresh dung, and as dung dries up, it quickly loses its attractiveness to dung beetles (Howden and Nealis 1975, Halffter and Edmonds 1982). Additionally to there being fewer and smaller beetles during the day, dung probably dries more quickly during the day than during the night, which would further diminish the probability of a seed being buried during the day. This was further corroborated by the fact that differences in dung removal after 12 hours vs. 24 hours were more pronounced for dung piles placed in the morning than for dung piles placed in the afternoon, and by the fact that after 12 h, diurnal dung piles were more likely to have some dung remaining than nocturnal samples. The importance of nocturnal dung beetles in burying the dung of herbivorous mammals has been noted in two additional Neotropical forests (Estrada et al. 1993, Feer 1999).

Regarding seed/bead burial depth, the effect of time of day was similar as with percentage of seed/bead burial. In the four day vs. night comparisons, seeds/beads were buried more deeply when placed in the afternoon than when placed in the morning, although only in two of the four cases were the differences statistically significant. This

result may also be due to the greater abundance of larger beetles during the night. Although great variation exists among dung beetle species with respect to the depth at which they bury dung (Hanski and Cambefort 1991a), at least one study has shown that larger beetles bury dung more deeply (Doube 1990). Alternatively, the larger burial depths reported at night could be due to high levels of competition. More dung beetles were attracted to dung during the night, and since most of these beetles bury the dung below the dung source, in order to diminish competition for nesting space some beetle species may increase their burial depths, thus partitioning the vertical space beneath the dung source (Hanski and Cambefort 1991a).

From a plant's perspective, burial depth is important because seeds are more likely to escape detection by seed predators with increasing burial depths (Johnson and Jorgensen 1981, Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999). Conversely, seeds that are buried too deeply may not be able to emerge as seedlings (see Chapter 4). Thus, although more seeds are buried when defecated by mammals in the late afternoon and are also more likely to escape seed predation due to increased burial depths, more of these seeds may also fail to emerge as seedlings. In this respect it may be advantageous for a plant species to have some of its seeds dispersed by diurnal mammals and some dispersed by nocturnal mammals. In this way, while many of the seeds dispersed during the night will benefit from low predation rates, many of the seeds dispersed during the day will benefit from increased seedling establishment.

As seed size increases, the advantage of being handled by the nocturnal assemblage of dung beetles, rather than the diurnal, probably also increases, since large seeds are

mostly buried by large beetles, which in turn are more abundant during the night. In this study, seeds of *Pourouma guianensis* (11 mm in length) and of *Pouteria durlandii* (27 mm) were only buried by beetles larger than 10 mm, while some *Helicostylis scabra* seeds (5 mm) were also buried by smaller beetles. Smaller seeds on the contrary, may benefit more when handled by the diurnal assemblage of beetles, which on average consists of smaller beetles that probably bury seeds less deeply. In accordance with the findings reported in other studies (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999), I found that small beads were buried more often than large beads, but they were also buried more deeply than large beads. Thus, a larger proportion of small seeds may be buried at depths that hinder seedling emergence when handled by the nocturnal dung beetle assemblage.

On the other hand, rodent seed predators in Neotropical forests are mostly nocturnal (Emmons and Feer 1990), thus if seeds are defecated during the day, they are less likely to be buried by dung beetles, but they are also less likely to encounter rodent predators immediately after being deposited. Consequently, being defecated during the day may leave enough time for dung beetles to remove most or all of the dung (even if seeds are not buried), thus reducing the olfactory cue that attracts rodents. For large seeds, however, being defecated during the day and night might both be advantageous. Seeds defecated at night will have higher probabilities of being buried and escape predation. On the other hand, seeds defecated during the day may have a higher probability of being scatterhoarded by rodents. In Neotropical forests large diurnal cavylike rodents, the agouti (*Dasyprocta* spp.) and the acouchi (*Myoprocta* spp.) seem to be the most important group of scatterhoarding rodents, and they preferentially hoard large seeds

(Hallwachs 1986, Peres and Baider 1997, Forget et al. 1998, Asquith et al. 1999). When rodents scatterhoard seeds but later fail to eat them, they also act as secondary seed dispersers for these seeds. Thus, as already proposed by Feer (1999), is it likely that as seed size increases the role of scatterhoaring rodents as secondary seed dispersers increases, and the role of dung beetles diminishes.

Season. In five paired comparisons, the percentage of seeds/beads buried was higher in the rainy months than in the dry months. However, only two of the comparisons were statistically significant. As mentioned above, season of the year did not have a significant effect on the number of beetles captured, or on their mean size. It only had an effect on number of species captured, which is likely less important in terms of seed burial. However, season of the year may affect seed burial in other ways. For example, it is probable that during the rainy season, the soils are on average softer than during the dry season, which in turn may facilitate dung and seed burial by dung beetles. Many studies have shown that soil texture and hardness are important factors affecting the composition of local dung beetle communities and their dung-burying behavior (Fincher 1973, Hanski and Cambefort 1991b, Giller and Doube 1994, Osberg et al. 1994, Davis 1996).

I also found that dung removal after 24 h was more complete during the wet season than during the dry season. This may be due to the dung drying out more slowly during the rainy season. Alternatively, dung could have more often been washed away by rain during the rainy season than during the dry season.

As already mentioned above, season is likely to have a much more conspicuous effect on secondary seed dispersal by dung beetles, in forests in which a more

pronounced seasonality in the dung beetle community occurs. Studies done in such forests, have also pointed out this possibility (Estrada and Coates-Estrada 1991, Andresen 1999), but no study until now has actually measured the effect of season on secondary seed dispersal by beetles. In tropical dry forests, in which dung beetles are almost completely absent during the dry season (Janzen 1983, 1986), there may be strong selection pressure for plants that have their seeds defecated by mammals, to produce their fruits during the wet season. Of course, environmental factors affecting seed germination and seedling survival (e.g. water availability) are probably more important under such circumstances, and it would be difficult to separate the different causal factors that may be responsible for the observed phenology patterns.

Amount of dung. The amount of dung surrounding seeds was found to be important in determining seed burial by dung beetles. Seeds were buried more often when surrounded by larger amounts of dung, likely as a consequence that larger amounts of dung attracted more beetles. Additionally, beads surrounded by more dung were buried more deeply than beads surrounded by less dung. Thus, as with "time of day" (see above), from a plant's perspective there may be a tradeoff between the positive aspect of being surrounded by more dung (more seeds are buried and thus are more likely to escape from predation) and the negative aspect (seeds are buried more deeply and thus seedlings are less likely to emerge). The size of dung clumps will greatly depend on several aspects of the mammal species that produces them, such as body size, defecation behavior (see Chapter 3), diet composition, and digestive physiology. Consequently, as with time of day, the tradeoff between negative and positive effects of amount of dung surrounding

the seeds, probably selects in favor of a plant species having a variety of different frugivores dispersing its seeds.

However, by knowing the size distribution of fecal clumps produced by different mammals one could assess the proportion of seeds in their defecations that will be secondarily dispersed by dung beetles, and the depths at which they are more likely to be buried. Since the size distribution of fecal clumps is a characteristic of the primary disperser, and since this characteristic can determine seed fate, it follows that in studies that compare the effectiveness of different seed dispersers (sensu Schupp 1993) this aspect should be considered (see Chapter 3).

In terms of seed size, only large mammals (> 8 kg) are able to swallow very large seeds (> 40 mm); these animals are also the ones likely to produce larger defecations. It follows that seeds swallowed by these mammals may then be surrounded by larger amounts of dung and thus have a higher probability of being buried. Also, since larger seeds are on average buried less deeply by dung beetles they may suffer less from deep burial, and selection pressures may actually favor dispersal by large vs. small frugivores, in order to be surrounded by more dung. This, in turn would select for larger seeds, since smaller mammals are not likely to swallow large seeds. However, as seed size increases the probability of seed-swallowing decreases, and the probability of seed-dropping and/or seed-spitting increases. Because of this one could expect to observe other mechanisms by which the plant encourages seed-swallowing of large seeds. For example, some plant species produce fruits in which the pulp is very firmly attached to the seed, and only through seed ingestion can the animal fully make use of the pulp available. For example, many Sapotaceae trees, have large indehiscent fruits which can only be manipulated by

arboreal mammals (or seed-eating parrots), and thus many of these species seem to be exclusively dispersed by mammals (Julliot 1994, Chapter 3). Moreover, many of these species also present a pulp that is firmly attached to the seed, promoting seed-swallowing and dispersal through defecation.

Horizontal movement of seeds. Dung beetles also move seeds horizontally, potentially diminishing the degree of seed clumping when several seeds are defecated together. The maximum distance recorded in this study was 60 cm with mean distances for different species varying between 6 and 17 cm. But still, small distances as these can have important implications for plant demography. Seedlings germinating from seeds in a clump will compete with each other, and only one seedling will usually survive (Howe 1989). However, seedlings that establish from seeds that are physically separated by even a few centimeters are likely to survive to the sapling stage. Thus, even short-distance horizontal movement of seeds away from each other will increase the number of individuals available for recruitment into the next age-class.

Other studies have reported greater maximum horizontal distances than those reported here, for dung and/or seeds moved by dung beetles in tropical forests, in particular by the "roller" dung beetles (Estrada and Coates-Estrada 1991, Andresen 1999). This is due to the dung-relocating behavior of rollers. Roller dung beetles make a dung ball and always move it away from the source before burying it. Tunnelers, on the other hand, make a burrow close to the dung, and make several trips to the dung source to provision their burrow (Halffter and Edmonds 1982).

This study, as well as others, have found that tunneler dung beetles in Neotropical forests are generally more abundant in terms of species and individuals (Peck and

Howden 1984, Estrada and Coates-Estrada 1991, Hanski and Cambefort 1991e, Andresen 1999, this Chapter) and are represented by species of larger sizes than rollers (Estrada and Coates-Estrada 1991, this Chapter). Additionally, tunnelers worldwide remove larger amounts of dung, from a dung source, than roller dung beetles of equivalent size (Doube 1990). Thus, tunnelers are likely to be, in general, the most important group of dung beetles in secondary seed dispersal, in terms of the percentage of seeds they bury. Rollers, however, may be very important in cases in which the advantages of reduced seed density are higher relative to the advantages of reduced seed predation.

In the present study I measured the horizontal distances that seeds were moved after dung beetle activity, but I did not record distances for individual beetle species. Also, some of the seeds/beads used in the experiments in this study were not found. Seeds that were not found were likely removed by seed predators (see Chap.4). However, in the case of beads that were not found (approximately 5 %), these were either buried together with the thread that was attached to it, and consequently I could not find them, or they were taken beyond the 5 m-radius area that I searched for moved/buried seeds. These 'lost' seeds/beads were not included in analyses. Thus, it is likely that I underestimated to some extent both the mean and maximum distance that beetles can move seeds.

In terms of the effect of amount of dung, unexpectedly, more seeds were moved horizontally when surrounded by less dung. This may indicate that more dung beetles remove dung away from the dung source and away from competitors when the resource is less abundant.

In conclusion, the forests in Central Amazonia hold a rich dung beetle community that plays an active role in the scondary dispersal of seeds that are defecated by

mammalian frugivores. The relationship between dung beetles and seeds, however, is not a simple one; many factors add complexity to this interaction. In this study I found that factors such as seed size, amount of dung surrounding the seed, time of day in which the seed is deposited, and season in which the seed is dispersed, can affect the immediate outcomes of seed-beetle interactions, and thus the short-term fate of seeds. The ultimate outcomes of these interactions, however, will depend on the long-term fate of the seeds moved by beetles, which is the focus of Chapter 4.

PRIMARY SEED DISPERSAL BY HOWLER MONKEYS AND THE EFFECT
OF DEFECATION PATTERN ON THE FATE OF DISPERSED SEEDS

Introduction

The majority of tree species in tropical forests produce fruits that are adapted for consumption and seed dispersal by frugivorous vertebrates (Howe 1990). The fruits of most of these plant species are eaten my more than one species of frugivore and consequently, the seeds of these plants are also usually dispersed by more than one species of animal (Andresen in press). What is important from the plant's perspective, however, is the effectiveness of a given disperser species. As defined by Schupp (1993), the effectiveness of a seed disperser is defined by the quantity of seeds dispersed and the quality of dispersal.

Animals can relatively easily be compared in terms of dispersal quantity -- one assesses the number of seeds dispersed during the fruiting season of a given plant. Far more difficult to assess is the quality of dispersal, since to do this the post-dispersal fate of seeds has to be determined. In some instances, ingestion of seeds significantly increases or decreases germination percentage and/or germination rate. This aspect of the dispersal quality can be determined with germination experiments. However, dispersal quality is also affected by many biotic and abiotic factors that are encountered by the seed after deposition. A high quality site of deposition is one in which the combination of biotic and abiotic factors favors high seed/seedling survival (Schupp 1993).

One aspect that can greatly affect the seed-dispersal quality of a frugivorous animal that disperses seeds through defecation, is also one that has received very little attention, the defecation pattern. Defecation patterns may vary greatly among different frugivores, according to features such as the size of the animal, its social/ranging behavior, and digestive physiology (Zhang and Wang 1995, Andresen 1999). In the case of seeds defecated by mammals, for example, many dispersed seeds are surrounded by fecal material, which is known to attract seed predators and secondary seed dispersers. More specifically, mammalian defecations attract dung beetles and rodents. Dung beetles bury seeds incidentally, while burying dung; rodents actively search for seeds in dung to feed on them (Janzen 1986, Chapman 1989, Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Chapters 2 and 4). Seeds buried by dung beetles have a high probability of avoiding rodent predation (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Chapter 4) but may suffer decreased seedling emergence if they are buried too deeply (Chapter 4).

Not just the presence of dung but also the amount of dung and the spatial pattern of dung/seed deposition may affect the behavior of rodents and dung beetles. For example, a high density of dung and/or seeds (aggregated dung- and/or seed- deposition pattern) may yield a stronger cue (mostly olfactory) for rodents and dung beetles than a low density (scattered dung- and/or seed- deposition pattern).

Thus, since frugivorous animals differ in the defecation patterns they produce, this aspect of primary seed dispersal should be taken into consideration when assessing the effectiveness of a given animal species as a primary seed disperser. This is particularly relevant in the case of seed species that are dispersed by several species of

frugivorous animals, and which will consequently have their seeds deposited in several ways. For example, seeds swallowed and defecated by large mammals, such as howler monkeys or tapirs, may be deposited in large aggregations of seeds and dung (e.g., Howe 1980, Bodmer 1991). On the other hand, seeds of the same species swallowed and defecated by smaller mammals, or mammals with different defecation behaviors, may be deposited singly or in small groups, surrounded by small amounts of fecal material (e.g., Andresen 1999). Finally, some seeds that are defecated may be deposited without fecal material when they separate from the dung while hitting vegetation layers as they fall to the ground (Ganzhorn et al. 1999). In some special cases seeds may be expelled from the intestine without fecal material (Garber 1986).

Another aspect in which different frugivorous animals can be compared in terms of their seed-dispersing activity, is their importance at the plant community level. Here I define an important disperser at the plant community level as one that disperses the seeds of many species. This aspect of seed dispersal may have practical implications for forest conservation and management. For example, it is sometimes necessary to determine which seed-dispersing species should become the focus of conservation efforts, when the overall goal is maintaining the regeneration ability of a forest fragment. Of course, an animal that disperses the seeds of many species, but whose effectiveness for each of the plant species is very low, will be of little ecological importance in terms of forest regeneration. On the other hand, a species that is a very effective disperser for only a few plant species, may not be the optimal disperser either. Ideally one would want to maintain healthy populations of animal species that are both important and effective dispersers.

One mammal species that has been recognized as a seed disperser in many Neotropical forests, is the howler monkey (Alouatta spp., e.g., Mittermeier and van Roosmalen 1981, Estrada and Coates-Estrada 1984, Galetti et al. 1994, Julliot 1996b). However, howler monkeys have sometimes been regarded as less important seed dispersers at the plant community level, and as less effective dispersers at the plant population level, than other primary dispersers (Howe 1980, Mittermeier and van Roosmalen 1981, Andresen 1994).

At the plant community level, howler monkeys in several forests have been reported to consume the fruits of not more than 35 species of plants (Milton 1980, Gaulin and Gaulin 1982, Estrada and Coates-Estrada 1984, Galetti et al. 1994, Andresen 1999), which is low compared to the fruit diet of other primates, such as spider monkeys (*Ateles* spp.) or capuchin monkeys (Cebus spp., Roosmalen 1985, Andresen 1994, Guillotin et al. 1994, Simmen and Sabatier 1996). However, there seem to be areas in which howler monkeys are consuming the fruits of many more plant species than reported in most studies. In French Guiana, for example, howler monkeys dispersed the seeds of over 100 species of plants (Julliot and Sabatier 1993, Julliot 1996a).

Also, howler monkeys are the only large arboreal mammals capable of surviving in small forest fragments (10 ha or less), and thus may play a crucial role in the regeneration of plants (large-seeded species in particular) in such remnant habitats (Neves and Rylands 1991, Ferrari and Diego 1995, Estrada and Coates-Estrada 1996, Chapter 5).

At the plant population level, howler monkeys are regarded as less effective dispersers, due to low dispersal quality (Howe 1980, 1986; de Figueiredo 1993). They are considered low quality dispersers for some plant species because their defecations result in large aggregations of seeds and dung on the forest floor. Such spatial distribution of seeds has been argued to be disadvantageous for plants due to density-dependent seed/seedling mortality in defecation sites. However, the fate of seeds dispersed by howler monkeys has not been followed, nor has it been compared to the fate of seeds dispersed in different spatial distributions. Further, the presence of fecal material around seeds dispersed by mammals has only been considered in a few studies that have assessed seed fates (Janzen 1986, Chapman 1989, Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999).

The main objectives of this study were to determine how many plant species are dispersed by the red howler monkeys in a Central Amazonian rainforest and to examine how different patterns of defecation influence the fate of dispersed seeds and, consequently, the quality of dispersal. Specific questions were:

(1) How many seed species do howler monkeys disperse?
(2) What are the patterns of dung- and seed-depositions produced by howler monkeys?
(3) What is the effect of presence of dung and dung/seed densities on seed predation by rodents?
(4) What is the effect of dung/seed densities on secondary dispersal by dung beetles?

In summary, this chapter helps to link the stages of primary seed dispersal and seedling establishment, by assessing how characteristics of seed deposition (defecation pattern) affect the fate of dispersed seeds. This is important because it defines another characteristic associated with the primary disperser that ought to be taken into

consideration when different species of dispersers are compared in terms of their seed dispersal quality.

Methods

Study Site

This study was conducted in a 10,000 ha forest reserve (reserve #1501, also known as "Km 41") situated about 90 km north of the city of Manaus (2°30'S, 60°W), in the Brazilian state of Amazonas. This reserve is part of the Biological Dynamics of Forest Fragments Project (BDFFP), which was formerly known as the Minimum Critical Size of Ecosystems Project (Lovejoy and Bierregaard 1990). Mean annual temperature and precipitation in Manaus are 26.7°C and 2,186 mm, respectively (Lovejoy and Bierregaard 1990). A relatively drier season occurs from June through October.

The forest is classified as tropical moist (Holdridge 1967). The reserve is mainly covered by *terra firme* (not subject to river flooding) forest. The canopy is 30-37 m tall (Lovejoy and Bierregaard 1990), and the most important tree families are Burseraceae, Sapotaceae, Lecythidaceae, and Leguminosae (Rankin-de-Merona et al. 1992). More detailed descriptions of BDFFP reserves can be found in Lovejoy and Bierregaard (1990), Prance (1990), and Rankin-de-Merona et al. (1992).

Primary Seed Dispersal and Defecation Pattern

I located howler monkeys when they vocalized at dawn (usually 0400-0600 h). Defecation typically occurs later in the morning, usually between 0700 and 1000 h, at which time I visited the site again and collected all seeds and dung I could find on the forest floor. Although dung beetles are rapidly attracted to dung and probably some of

the dung had been removed by them when I collected it, I do not believe that the amount of dung removed by dung beetles constituted a significant proportion of the defecation samples. Moreover, diurnal dung beetles are less abundant and of smaller mean size than nocturnal beetles, and dung removal is significantly slower during the day than during the night (see Chapter 2).

From May 1996 to May 1998, 263 defecation samples were collected. Whereas in other studies authors have used the term defecation for one single fecal pile and usually refers to the dung defecated by one individual, here I define defecation sample as all the dung found on the forest floor produced after one or more howler monkeys had defecated and the whole troop had moved to a different area. Consequently each defecation sample in this study consisted of several to many individual fecal piles. Although usually most members of a howler monkey troop will defecate simultaneously from the same tree or neighboring trees (Andresen 1994, 1999; Julliot 1996b), sometimes only one or two individuals defecate in a site, and the troop travels to a different site before the rest of the troop defecates (Andresen 1994).

Red howler monkeys have a bimodal defecation pattern, in which most members of a troop defecate simultaneously twice a day: once in the morning before beginning the days activities, and once in the afternoon, at the end of a long resting period in the middle of the day (Andresen 1994, Julliot 1996b). As a consequence, 61% of all defecation occurs around resting areas (Julliot 1996a). Therefore, although I only collected defecation samples in the morning, I consider these samples to be representative of the defecation pattern produced by red howler monkeys.

Most defecation samples were collected from five howler monkey troops (4-9 individuals in each) with home areas closest to the research station. Occasionally, defecation samples were also collected from other troops. Defecation samples were taken to the research station and all seeds > 3 mm were removed by hand (using gloves). Seeds > 3 mm were removed to avoid interference of these seeds with experimental seeds used in the experiments described below. Seeds ≤ 3 mm were not common in defecation samples (only 8% of all defecation samples contained seeds ≤ 3 mm, including *Ficus* spp.seeds), their removal was not practical, and they were considered unlikely to affect the interaction between dung beetles and experimental seeds).

Freshness of dung is important for its attractiveness to dung beetles (Howden and Nealis 1975, Halffter and Edmonds 1982). Therefore, the seedless dung was stored in plastic bags and used in experiments the same day. When the dung could not be used the same day (e.g., due to rain), it was stored in an insulated box with ice and used the following day. Seeds were washed and counted and a sample of seeds for each species was randomly chosen and the seeds measured (length, width, and thickness/height). Seed samples were placed in plastic bags with alcohol (70%) and taken to M.G.M. van Roosmalen at the INPA (National Institute for Research in Amazonia) Botany Department (Manaus) for identification. At the end of the study period, the samples were deposited in van Roosmalen's seed collection.

Total fresh weight of the defecation samples was recorded for 205 of the samples. One hundred thirty defecation samples (samples with no seeds ≤ 3 mm), were weighed before and after removal of all the seeds, to estimate the proportion of the total

defecation-sample weight that was due to seed weight vs. non-seed fecal material. For 34 defecation samples I estimated the total area of forest floor in which dung and seeds had fallen. For 30 defecation samples I counted the total number of individual dung piles that constituted the whole sample of known weight and for 13 of these defecation samples I weighed individual dung piles (all these samples had very few or no seeds in them). Finally, for nineteen defecation samples that contained seeds > 3 mm I counted, by species, the seeds that fell on the ground surrounded by dung and the seeds that had been separated from the dung while falling to the ground.

Effect of Defecation Pattern on Rodent and Dung Beetle Behavior

    Experiment 1: Seed predation by rodents: the effect of presence of dung and of dung/seed pile density. In this experiment I assessed seed predation pressures on seeds mimicking four types of seed deposition patterns by frugivorous animals: high seed and dung densities, high seed density without dung, low seed and dung densities, and low seed density without dung.

    To assess seed predation pressures I used piles with five raw sunflower seeds each, placed on the forest floor. I used sunflower seeds because they could easily be bought in the market, they could be stored, and rodents are known to feed on them. Since I only wanted to assess the relative predation pressures in the different treatments, it was not important what kind of seed I used as long as it was consumed by seed predators. So, I chose a species that is likely to be palatable to most rodent predators. I used 5 seeds per pile to increase probability of detection by seed predators. The seed piles were placed in four treatments:

(A) High seed and dung densities: five piles of seeds spaced evenly in a 2 x 2 m area in the middle of a howler monkey defecation site. The sunflower seeds were placed immediately after the howler monkey troop had finished defecating and I had removed all of the seeds and most of the dung. Next to each seed pile I placed 10 g of dung and ca. 50 additional g of dung were left on the surface (approximate dung density of 25 g/m^2, which is similar to the median dung density observed in howler monkey defecation samples: 31 g/m^2).

(B) High seed density without dung: five piles of seeds spaced evenly in a 2 x 2 m area, with no dung, approximately 30 m away from treatment A.

(C) Low seed and dung densities: a single pile of seeds placed on the forest floor next to 10 g of howler monkey dung. Not in or near a howler monkey defecation site.

(D) Low seed density without dung: a single pile of seeds without dung.

Treatment A was placed in a howler monkey defecation site, immediately after the group had defecated. This was done because I had observed that some diurnal rodents (e.g. agoutis and acouchis) often visited howler defecation sites soon after defecation had occurred, as if they had been "monitoring" the monkeys. Treatments A and B were placed simultaneously. Treatments C and D were placed on a transect approximately 30 m away from the defecation site, two or three days after treatments A and B had been placed. Treatments C and D were set out simultaneously on the transect, one seed pile every 10 m, alternating treatments and using 5 seed piles/sample for each C and D (100 m total transect length). Sample size for each of the four treatments (A, B, C and D) was 13.

The number of seeds remaining in each seed pile was recorded after one day. One day was used because it is during the first 24 h that the difference among treatments in terms of presence of dung is most pronounced.

Experiment 2: Bead burial by dung beetles: single vs. aggregated dung piles.
To assess the activity of dung beetles in aggregated vs. scattered defecations, round plastic beads (8 mm diameter; bead # 4 from Chapter 2) were used as seed mimics. I

used 10 g dung piles, and placed one bead inside each of the piles. I placed bead-containing dung piles in two treatments, simulating two different defecation patterns produced by mammals: (i) an aggregated pattern similar to the one produced by howler monkeys, with ten dung/bead piles in an area of 2 x 2 m (total dung density: 25 g/m^2; N = 16 groups, one or two groups set out on any single day, if two groups were set out they were separated by a distance of at least 50 m); and, (ii) a scattered pattern with individual dung/bead piles along transects, one pile every 10 m (80 single dung/bead piles were used). Although treatments were not set out simultaneously, they were all set out during the dry season and seeds for both treatments were placed in the same general area of the forest. The data for the scattered treatment come from Experiment 1 of Chapter 2.

A 50 cm-long piece of white nylon thread was attached to each bead, to find the beads that were buried by dung beetles. After two days each location where a dung/seed pile had been placed was checked and it was recorded whether the bead had been buried by dung beetles (checking was done after two days to allow all dung to disappear). Buried beads were carefully unburied and burial depth was measured to the nearest centimeter (when beads were slightly covered by soil, a burial depth of 0.5 cm was assigned.

Experiment 3: Seed burial by dung beetles: effect of seed density and amount of dung in single dung piles. To assess the effects of seed density and amount of dung in each dung pile, I used two states of each of these variables, placing experimental seeds in each of the following combinations: (i) one seed in a 10 g dung pile; (ii) four seeds in a 10 g dung pile; (iii) one seed in a 25 g dung pile; and (iv) four seeds in a 25 g dung pile.

The amounts of dung used are within the range of sizes produced by howler monkeys as well as other fruit-eating mammals, without representing extremes (Estrada et al. 1993, Andresen 1999, this Chapter). Experimental seeds were placed along transects, one dung pile with seed(s) every 10 m. Seeds in each of the four dung-amount/seed-density combinations were set out in the same general area and during the same season. For the 10 g dung treatment I used 154 piles with single seeds and 61 with groups of four seeds; for the 25 g dung treatment I used 123 piles with single seeds and 93 with groups of four seeds.

Seeds of *Byrsonima crispa* (Malpighiaceae) were used (spherical seeds 8.1 ± 0.2 mm diameter, N = 15; here and elsewhere I report mean ± one standard error). All seeds were marked with a 50 cm long nylon thread glued to them. After two days, I counted the number of seeds buried by beetles. Burial depths were measured after two days for some of the seeds and after a year for others. This is because some seed transects were set out with the purpose of following seed fate until germination, and thus they were not unburied on the second day. However, seeds suffered no seed predation and, while they remained viable, they did not germinate during the study period (I found out later that seeds of this species need high light levels to germinate).

Data Analysis

Results on primary dispersal and defecation pattern are expressed as mean numbers and mean percentages in the case of normally distributed variables, and as median numbers and median percentages in the case of non-normally distributed variables. A Pearson Chi-square test was used to test for independence between the size of seeds (< 15 mm and ≥ 15 mm long) and frequency of defecated seeds that were surrounded by dung

vs. defecated seeds that were free of dung. The size categories were chosen according to the mean length of seeds swallowed by howler monkeys which was 15.5 mm (see below).

Data on seed predation/removal of sunflower seeds (Experiment 1) were analyzed with a one-way ANOVA and pairwise post-hoc comparisons among the treatments using Bonferroni adjusted probabilities.

Regarding seed burial by dung beetles in single dung piles vs. aggregated dung piles (Experiment 2), single seeds were pooled in groups of 10 consecutive seeds to allow comparison with the "aggregated" treatment. The percentage of seeds buried in each group of 10 seeds was calculated and differences among treatments were tested with a two-tailed t-test. No data transformation yielded a normal distribution of burial depth, thus, differences in this variable among treatments were tested with a two-tailed Mann-Whitney U test. In this case sampling units were individual seeds.

Finally, data from Experiment 3 were analyzed with two two-way ANOVA's to test for the effect of seed density (1 vs. 4 seeds in isolated dung piles), dung amount (10 g and 25 g dung piles), and their interaction, on the percentage of seeds buried (transformed as the arcsin square-root of the proportion of seeds buried) and on the depth of burial (square-root-transformed). To perform the ANOVA on percentage of seed burial, seed piles were pooled so that each sample for analysis had 8 seeds, i.e. in the treatment with 1 seed, 8 piles were pooled, while in the treatment with 4 seeds, two piles were pooled. Pooling in this manner facilitated the normal distribution of the variable.

Results

Primary Seed Dispersal

One hundred and thirty-seven different species of seeds were found in the defecation samples of howler monkeys during the 25 month study period (N = 263 defecation samples). Sixty-four percent of all defecation samples contained seeds, while 36% had no seeds. Seasonal variation existed in the amount of fruit eaten and seeds dispersed, with a tendency towards higher seed densities at the end of the dry season and beginning of rainy season (Fig. 3.1). Large variability also existed between the first and the second year. Sixty-one percent of defecation samples collected during the first year (May 1996 - April 1997, N = 137 total defecation samples for first year) contained no seeds in them (most seed-less defecation samples were collected between May and November 1996). In the second year (May 1997 – May 1998, N = 126 total defecation samples for second year) only 9% of the defecation samples had no seeds in them (seed-less defecation samples were collected between May and September 1997).

Not all the species eaten by howler monkeys could be identified. Of the 92 species that were identified at least to the family level, 61% (56 species) belonged to the Sapotaceae, 14% (13 species) to the Moraceae, 7% (6 species) to Leguminosae. The rest were distributed among 13 other plant families. Measures of seeds were taken for 112 of the species. The mean length of seeds swallowed by howler monkeys was 15.5 ± 0.58 mm and the mean width was 9.9 ± 0.32 mm; the maxima were 32.5 mm and 17.8 mm for length and width, respectively. The average size of the 47 species of Sapotaceae seeds measured was above the average of all the species measured. Mean
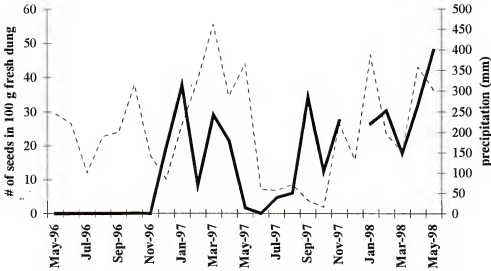
Figure 3.1. Monthly rainfall (dotted line) and abundance of seeds found in howler monkey defecations (solid line) during the study period (no defecations were collected during December 1997).
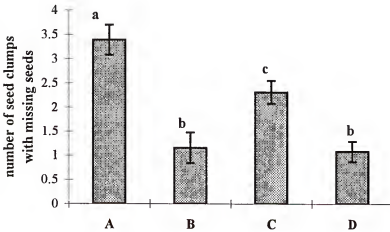
Figure 3.2. Mean number of sunflower seed clumps, out of 5 seed clumps, with at least one seed missing in four seed/dung patterns: (A) high seed and dung densities (5 seed clumps with 10 g of dung next to each seed clump), (B) high seed density (5 seed clumps) without dung, (C) low seed and dung density (1 seed clump with 10 g of dung next to it), (D) low seed density (1 seed clump) without dung (see text for a more detailed explanation of treatments). Error bars represent standard error; N = 13 for each treatment. Matching letters above bars indicate statistically homogeneous samples (P > 0.05), while different letters indicate statistical differences (P ≤ 0.05).

length of Sapotaceae seeds was 19.6 ± 0.69 mm and mean width was 11.2 ± 0.38 mm; the minimum size of Sapotacea seeds was 11 x 6 mm.

Defecation Pattern

Howler monkey defecation samples (defined above as all the dung produced by one or more howler monkeys falling on the forest floor) weighed on average 408 ± 17.7 g. Most of the variation observed in the total weight of defecation samples (Table 3.1) was due to the fact that although most often (ca. 75% of the times) all or most of the members of a troop defecate at approximately the same time, it is not uncommon that only one or a few individuals will defecate at a given moment, while the rest of the troop will defecate after travelling for some distance (personal observation). Consequently, also the area in which the defecation samples fell varied greatly (Table 3.1) and showed a median of 17 m^2. The number of separate dung piles that constituted a defecation sample averaged 62 ± 5.4. The median amount of dung per square meter in a defecation site was 31 g and the median number of dung piles per square meter was 6. The median weight of individual dung piles was only 2.5 g (N = 796). However, 48% of all dung collected was made up of piles that weighed 5-30 g, while 29% of all dung was made up of piles > 30 g and 23% of piles < 5 g.

In those defecation samples in which seeds > 3 mm were present, seeds accounted for an average of 37 ± 1.7 % of the total weight of the defecation sample (Table 3.1). The median number of different seed species found in seed-containing defecation samples was 4. The median number of seeds > 3 mm found in 100 g of dung was 23, with a median total of 67 seeds dispersed in a defecation sample. An average of 19 ± 2.4 % (N = 19) of all the seeds in a defecation sample were not surrounded by dung,

but separated from it in the process of falling to the ground. Larger seeds (≥ 15 mm long) were separated from the non-seed fecal material more often (19%, N = 2175 seeds from 19 defecation samples) than smaller seeds (< 15 mm long, 11%, N = 592 seeds from 19 defecation samples; $\chi^2 = 22.26$, d.f. = 1, P < 0.01). The percentage of seeds that separated from the fecal material did not depend on seed density in of defecation samples (linear regression, $F_{1, 17} = 0.00$, P = 0.99).

Table 3.1. Characteristics of the dung- and seed-depositions produced by howler monkeys (one or more individual monkeys defecating simultaneously).

Variable	Total	Mean ± 1 SE, or Median	Range	Sample size
Seed species defecated	137			263
Defecation samples without seeds	36%			263
Defecation sample weight		408 ± 17.7 g	30 – 1140 g	205
Dung weight/m²		31 g	1 – 211 g	34
Defecation sample area		17 m²	1 – 63 m²	34
Dung piles/defecation sample		62 ± 5.4	8 – 112	30
Dung piles/m²		6	0.3 – 28	30
Dung in piles of 5-30 g	48 %			4771.5 g
When seeds > 3 mm were present in defecation samples:				
Seed weight in a defecation sample		37 ± 1.7 %	0.3 – 77 %	130
Seed species/defecation sample		4	1 – 12	154
Seeds/defecation sample		67	1 – 560	154
Seeds/100g dung		23	0.2 – 137	154
Seeds without dung/defecation sample		19 ± 2.4 %	0 – 35 %	19

Effect of Defecation Pattern on Rodent and Dung Beetle Behavior

Experiment 1: Seed predation by rodents: the effect of presence of dung and of dung/seed pile density. Significant differences were observed in the amount of seed

predation among the four dung/seed deposition patterns (one-way ANOVA, $F_{3, 48} =$ 16.02, $P < 0.01$). Predation was highest for aggregated seed piles in defecation sites (treatment A: high seed and dung densities), followed by single seed piles placed next to 10 g of dung (treatment C: low seed and dung densities) and was lowest for seed piles with no dung, regardless of the density of seed piles (treatments B and D: high seed density without dung and low seed density without dung, respectively; Fig. 3.2). All Bonferroni-adjusted post-hoc pairwise comparisons were statistically significant (P's $\leq$ 0.05), except the comparison between the two treatments without dung ($P > 0.1$).

Experiment 2: Bead burial by dung beetles: single vs. aggregated dung piles.
No significant differences were found in the percentage of beads buried by dung beetles or burial depth in single vs. aggregated dung piles (Fig. 3.3). An average of 46% of beads in aggregated dung piles were buried, while 53% of beads in single dung piles were buried (t-test, $T = -0.82$, d.f. = 22, $P = 0.42$). Beads in aggregated dung piles were buried at an average depth of 38 mm, and beads in single dung piles were buried at 34 mm (Mann-Whitney U test, $U = 1482.50$, $N = 69$ for aggregated and 41 for single piles, $P = 0.67$).

Experiment 3: Seed burial by dung beetles: effect of seed density and amount of dung in single dung piles. In 10 g dung piles, 57% of single seeds (1 seed/pile) were buried and 59% of grouped seeds (4 seeds/pile) were buried at average depths of 43 mm and 56 mm, respectively. In 25 g dung piles, 77% of single beads were buried and 73% of grouped seeds were buried at average depths of 69 mm and 73 mm, respectively (Fig. 3.4). No effect of seed density was found for either percentage of seeds buried or burial depth; similarly the interactions between seed density and dung amount were not
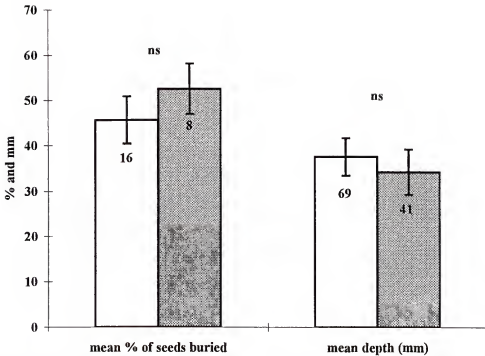
Figure 3.3. Mean percentage of beads buried and mean burial depth for beads in aggregated (white bars) and single dung piles (grey bars). Error bars are 1 standard error. Sample sizes are given inside bars. Sample sizes for % burial represent number of groups of 10 seeds pooled; sample sizes for burial depth represent individual seeds. "ns" indicates that the comparisons are not statistically significant (P > 0.05)
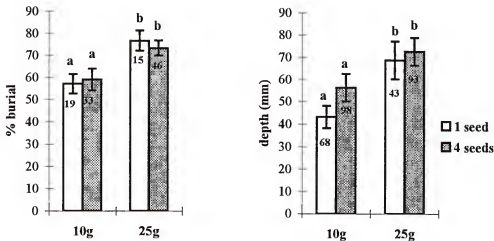
Figure 3.4. Percentage of seeds buried and burial depth for seeds placed in groups of four and singly, inside 10 g and 25 g dung piles. Error bars indicate standard error. Numbers inside bars are sample sizes, which are groups of 8 pooled seeds in the case of % burial, and single seeds in the case of burial depth. Matching letters above bars indicate statistically homogeneous samples (P > 0.05), while different letters indicate statistical differences (P ≤ 0.05).

significant; dung amount had the only significant effect on both variables with seeds being buried more often and deeper when deposited inside larger dung piles (2-way ANOVA, for percentage of seeds buried: density, $F_{1,109} = 0.02$, $P = 0.88$; dung amount: $F_{1,129} = 11.86$, $P < 0.01$; density x dung amount: $F_{1,129} = 0.28$, $P = 0.60$. For burial depth: density, $F_{1,298} = 0.81$, $P = 0.37$; dung amount: $F_{1,298} = 10.43$, $P < 0.01$; density x dung amount: $F_{1,298} = 0.30$, $P = 0.58$).

Discussion

Primary Seed Dispersal by Howler Monkeys

Similar to what has been reported for howler monkeys in other regions (e.g., Milton 1980, Mittermeier and van Roosmalen 1981, Estrada and Coates-Estrada 1984, Galetti et al. 1994, Julliot 1996b) great variability was observed in the amount of seeds found in the feces in different months, with long periods in which no seeds were found in the dung. Gilbert (1994) studied four howler monkey troops in the same study site in Central Amazonia during two years (1991-1993) and found that on average they spent 57% of their time eating fruit (great variability between months was also observed). This is among the highest percentages of fruit-eating recorded for an *Alouatta* species (see review in Julliot and Sabatier 1993).

Yet, what is even more unusual in this study site in Central Amazonia, is the extremely high number of different plant species that are swallowed and defecated by howler monkeys. I found 137 seed species in howler monkey defecation samples over a 25 month period. Most studies on the diet of howler monkeys report fruit diets consisting of 12 to 34 species (Milton 1980, Gaulin and Gaulin 1982, Estrada and

Coates-Estrada 1984, Galetti et al. 1994, Andresen 1999). Only in French Guiana do howler monkeys disperse through defecation a number of plant species similar to the one observed in this study: 110 species (Julliot and Sabatier 1993, Julliot 1996b).

These differences are no doubt due in part to differences in forest composition among the study sites. Forests in Central Amazonia are more similar in composition to forests in Eastern Amazonia and the Guianas, than to forests in Western Amazonia or Central America (Terborgh and Andresen 1998). Thus, it is not surprising to find similar diets for the howler monkeys in the Manaus area and in French Guiana. Additionally, the presence and abundance of competing species of arboreal mammals likely differs among sites. Howler monkeys are easily displaced from fruiting trees by other species, such as the spider monkey (*Ateles* spp., personal observation), and probably exploit some fruit species less frequently in sites where competitors are in high densities. In my study site, spider monkeys are extremely rare (Rylands and Keuroghlian 1988, personal observation) and this may favor fruit consumption by howler monkeys. Finally, fruit availability can vary tremendously from one year to another (personal observation) and even when fruit availability is similar in different years, primates may still show considerable variation in the species of fruits eaten and the proportion of the diet constituted by fruit (Chapman 1989). Thus, studies that examine the diet of a primate species during periods of one year or less, are likely to show a diet consisting of fewer plant species than longer studies. In my study, for example, howler monkeys only dispersed the seeds of 47 plant species during the first year (May 96 – April 97); 90 new species were added to the fruit diet in the second

year. Also, 61% of the 137 defecation samples during the first year contained no seeds, while only 9% of 126 defecation samples during the second year were seedless.

Howler monkeys are able to swallow large seeds (in this study: maximum length: 33 mm, maximum width: 18 mm). Similarly, in French Guiana, howlers swallowed seeds that were 40 x 25 mm in size (Julliot 1996b). Large seeds are only swallowed by large frugivorous animals. Moreover, while on the parent tree, fruits with hard indehiscent husks are likely to be eaten only by arboreal mammals, because they, unlike birds (except parrots, which are seed predators), have the manipulative and biting ability to open such fruits. Most Sapotaceae species have both characteristics: large seeds (> 10 mm long) and hard, indehiscent husks. As already noted by Julliot (1996b), howler monkeys and other large primates are probably the main seed dispersers of many Sapotaceae species and may thus be of particular importance for the regeneration of species in this plant family. In my study site, Spironello (1999) studied the assemblage of animals feeding on the fruits of nine Sapotaceae species. He found that the seeds of only one of the species were occasionally dispersed by toucans and that all other seed dispersal was accomplished by arboreal mammals, most importantly howler and capuchin monkeys (*Cebus apella*). In French Guiana and in my study site in Central Amazonia, Sapotaceae is one of the most important families of trees, both in terms of number of species and in density of trees (Rankin-de-Merona et al. 1992, Julliot 1996b). Also, Sapotaceae is the most important family in the fruit diet of howler monkeys in both forest sites. In French Guiana, 21 species of Sapotaceae were dispersed by one troop of howler monkeys (Julliot 1996b), while in my study site 56 species of Sapotaceae were found in defecation samples of several howler monkey

troops, with an average seed size of 20 x 11 mm (minimum: 11 x 6 mm). Since spider

monkeys are very rare in my study area (Rylands and Keuroghlian 1988, personal

observation) and capuchin monkeys only swallow smaller Sapotaceae seeds (personal

observation), howler monkeys may be the single most important seed disperser for

many Sapotaceae species in this forest in Central Amazonia.

Defecation Pattern

Defecation by howler monkeys probably produces one of the largest aggregations

of dung observed among Neotropical arboreal mammals. Due to their habit of all or

most members of a troop defecating simultaneously and from the same tree or

neighboring trees (Julliot 1996b, Andresen 1999), most of the defecation samples

contain a large amount of fecal material in a relatively small area of forest floor

(average of 408 g fresh weight of dung in 17 m^2). Also, due to their large body size (7-9

kg) and the large amount of leaves in their diet, howlers probably produce the largest

individual dung piles, compared to other arboreal frugivorous mammals (Estrada et al.

1993, Andresen 1999). So, although the median size of individual piles was only 2.5 g,

48% of all dung was in dung piles that weighed 5 – 30 g and 29% was in dung piles

that weighed > 30 g.

Large dung piles might be particularly advantageous to large seeds. Since large

seeds in a defecation sample were found to be more likely to separate from the dung

than smaller seeds, it is likely that large fecal clumps will contain large seeds more

often than small clumps. Why should it be more advantageous for a seed to be inside a

fecal clump than to fall loose? Only seeds in fecal clumps have a chance of being

buried by dung beetles and may thus avoid detection by seed predators (Johnson and

Jorgensen 1981, Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999, Chapter 4). Moreover, seeds in dung are more likely to be buried when surrounded by larger amounts of dung than when surrounded by smaller amounts of dung (Chapter 2).

Also, even just by being inside a dung pile, a seed may avoid predation, given that the dung pile is large enough to "hide" the seed (which will depend on the size of the dung pile relative to the size of seeds). Janzen (1982b), for example, found that seed-eating rodents were more likely to remove seeds from dung if the seed density in the clump was high and seeds were visible on the dung's surface. Thus, since the fecal material attracts seed predators (Janzen 1982b,1986; Andresen 1999; and see below), it may be that the most visible seeds in a defecation, i.e. seeds that are surrounded by small amounts of dung or that separate from the dung, are the ones that suffer the greatest predation rates, compared to seeds "hidden" in a large dung clump.

Although most of the defecations produced by howler monkey groups are large and clumped in space, some are not. It is not rare that only a single or a few individuals will defecate at the same time, producing a much smaller defecation sample than usual. I found, for example, that 26% of the defecation samples weighed less than 200 g and were thus probably produced by one or two individuals (estimated from Julliot 1996b, who calculated that a troop of six individuals defecated 1.5 kg of dung per day, in two defecations).

Yet another scenario occurs in which seeds may be dispersed in a scattered fashion by howler monkeys, regardless of the pattern of dung deposition. This happens when howler monkeys are eating a large amount of leaves relative to fruit. In such

cases, even though large defecation samples are produced, seed density may be very low. I found, for example, that although up to 560 seeds > 3 mm could be found in a single defecation, 20% of the defecation samples that contained seeds, had < 30 seeds. Unlike other primate species, howler monkeys swallow the seeds of most of the fruits they eat, rather than dropping or spitting them. For example, in the Peruvian Amazon only for one fruit species out of 15 consumed, were the seeds dropped rather than swallowed (Andresen 1999). Similarly, in French Guiana, howler monkeys only dropped the seeds of three species out of 90 fruit species consumed (Julliot 1996b). Thus, when few seeds are found in the dung of howler monkeys it generally means that they are not eating much fruit at that time.

When fewer seeds are present in a defecation, individual dung piles contain relatively more non-seed fecal material (Table 3.1), which is of course what dung beetles are attracted to. And, since larger individual dung piles attract more dung beetles, and present a higher probability of secondary seed dispersal, it follows that it may be advantageous for a plant species to produce ripe fruit during a time in which less fruit is available in the community. In tropical forests this time generally coincides with the dry season (Foster 1984, Terborgh 1986b). In this study I found during the dry seasons howler monkeys dispersed the seeds of relatively few species of plants, and furthermore relatively lower numbers of seeds were found per defecation sample, compared to samples in the rainy seasons. Of course, this may only be advantageous in forests in which dung beetles are also abundant during the dry season (Chapter 2).

Effect of Defecation Pattern on Short-Term Fate of Dispersed Seeds

The experiment with sunflower seeds clearly showed that dung attracts seed predators. Seeds in piles placed without dung suffered similar predation rates regardless of the density of seed piles (5 piles vs. 1 pile) and significantly lower predation than seeds with dung, both in single piles and groups of piles. In the case of seeds with dung, predation was lower for single seed piles than for groups of seed piles. Since the density of seed piles did not have an effect on predation rate in the treatments with no dung, I assume that the higher predation rate suffered by groups of seed piles with dung, when compared to single piles with dung, was due to the larger amount of dung or a combined effect of higher seed and dung density, rather than to seed density alone. This coincides with previous studies that also showed the effect of fecal material in attracting seed-eating rodents (Janzen 1982b, 1986, Chapman 1989, Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999).

However, dung is completely removed within one or two days by dung beetles and/or rain. Thus, although seed predation is higher when seeds are surrounded by dung vs. clean seeds and also in seeds with more dung vs. seeds with less dung during the first days after seed deposition, with time the effect of dung tends to disappear. Consequently, overall seed predation is likely to be similar for seeds with more dung, seeds with less dung and seeds with no dung (unless germination occurs very rapidly, Andresen 1999, Chapter 4).

In terms of seed density, some studies have shown increased seed removal by predators with increasing seed density (Janzen 1982b, Hulme 1994, Hammond 1995, Cintra 1997, Sánchez-Cordero and Martínez-Gallardo 1998), while others have found

no effect of seed density (Heithaus 1981, Webb and Willson 1985, Chapman 1989, Gryj and Domínguez 1996, Cintra 1997, Harrington et al. 1997, Sánchez-Cordero and Martínez-Gallardo 1998) and still some have reported higher survival probabilities for seeds in high densities (Janzen 1982a, Willson and Whelan 1990). These differences in results are likely due to differences in rodent behavior, which in turn are a consequence of many factors such as: rodent species, seed species, habitat, season, and overall fruit availability. In the case of most seeds defecated by mammals, seeds in high densities may often be surrounded by a high density of non-seed fecal material. Under this scenario, the dung attracts rodents to the defecation site and once there, if a rodent encounters seeds, it is likely to search the whole defecation area trying to find more seeds. And, since most of the seeds will have a "flag" of odorous fecal material around or next to it, it becomes an easy task for a rodent to find many of those seeds. Under this scenario, seeds well hidden inside large fecal clumps have perhaps the highest probability of survival, either because predators do not detect them or because dung beetles are more likely to bury them (see above).

Since buried seeds have a much higher chance of avoiding predator detection than seeds on the ground surface (Crawley 1992), defecated seeds that are buried by dung beetles have a higher chance of escaping predation (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999, Chapter 4). Moreover, rapid removal of the fecal material by dung beetles can make the defecation site less conspicuous to rodents and enhance the survival probability of seeds remaining on the surface. Large defecation samples, like those produced by howler monkeys, take longer to disappear than small defecation samples. Even after all the dung has been buried by

dung beetles (1-2 days), a strong odor remains in the area for one or two more days (personal observations), which may continue attracting seed predators. In this respect it is probable that seeds defecated by howler monkeys in large aggregations suffer higher predation rates than seeds in smaller defecations, if they are not buried by dung beetles.

Unlike with rodent behavior, the density of dung clumps did not have an effect on the behavior of dung beetles. Seeds in single dung piles (one dung clump with one seed) and seeds in aggregated dung piles (10 dung clumps in 2 x 2 m, with 1 seed each), had the same probability of being buried by dung beetles and were buried at the same depths. Similarly, seed density in individual dung piles (1 vs. 4 seeds in single 10 g-dung piles) had no effect on percentage of seed burial, or on burial depth. However, a significant effect was observed due to the size of individual dung clumps. Seeds were buried more often and more deeply when surrounded by 25 g of dung, than by 10 g of dung. The effect was the same for both seed densities (1 seed/dung pile and 4 seeds/dung pile). What this means is that while dung beetles are not responding to total dung amount in a defecation site (1 x 10 g vs. 10 x 10 g = 100 g in the comparison between single vs. aggregated dung piles), they are responding to dung amount in individual fecal piles. I found that pitfall traps baited with more dung, captured more beetles than traps with smaller baits (Chapter 2), which may explain the higher probability of a seed being buried if surrounded by more vs. less dung. However, in terms of defecation samples composed of more than one fecal clump, it seems possible that while more beetles are attracted to larger defecation samples (more total dung), the relative number and composition of dung beetles attracted per unit of dung is the same as in smaller defecation samples. A similar result was obtained by Feer (1999) in

French Guiana. He observed the same dung removal rates (after 8-10 and 24 h) for large quantities of dung (300-800 g) under howler monkey resting areas, and for individual 50 g dung piles.

What these results imply, in terms of the effect of defecation pattern on seed fate, as determined by dung beetle activity, is that seeds defecated by mammal species that produce defecations differing in the total amount of dung and/or seed density, may have similar secondary dispersal by dung beetles, as long as the size distribution of individual fecal clumps is also similar. However, dung beetles interact with rodents to determine the fate of the seed, and, as we have seen, rodent behavior is affected by the total amount of dung, or an interaction of dung amount and seed density, in a defecation sample.

Concluding Remarks on Primary Seed Dispersal by Howler Monkeys in Central Amazonia

Howler monkeys seem to be important seed dispersers at the plant community level in this rainforest in Central Amazonia. Because spider monkeys, the other large frugivorous primate in the area, is very rare, howler monkeys may be particularly important for large-seeded plants that are not dispersed by smaller mammals or birds. Such is the case of many species of Sapotaceae, which is a dominant family of trees in this forest.

At the plant population level, seed dispersal by howler monkeys often produces large aggregations of seeds and dung on the forest floor. Dung attracts rodents that feed on seeds and dung beetles that bury them. Seeds dispersed in such a way are likely to suffer higher predation rates than either seeds dispersed with dung in a scattered pattern

or seeds dispersed with no dung, but only if they remain on the surface. If, on the other hand, they are buried by dung beetles, probabilities of seed survival are enhanced tremendously (Chapter 4). Seeds have a higher probability of being buried by dung beetles if they are in large fecal clumps than if they are in small clumps. In this context, it may be that seeds dispersed by howler monkeys, who likely produce larger individual fecal clumps than most frugivorous Neotropical mammals (e.g., Estrada et al. 1993, personal observation), have higher probability of being buried by dung beetles, and thus better chances of survival. Large-seeded species in particular might benefit from being in large fecal clumps as compared to small fecal clumps.

CHAPTER 4.
SECONDARY SEED DISPERSAL BY DUNG BEETLES: THE EFFECT ON LONG-TERM SEED FATE AND SEEDLING ESTABLISHMENT

Introduction

Understanding processes affecting initial stages in the life history of plants is crucial for explaining and predicting forest structure. Because most of the mortality in the life cycle of plants occurs during the early phases of seed dispersal and seedling establishment (Harper 1977, Fenner 1987), any process that conveys a survival advantage to seeds and/or seedlings has the potential of increasing a plant's fitness. Seed dispersal has long been recognized, in both theoretical and empirical studies, as being such a process (see reviews in Howe and Smallwood 1982, Dirzo and Domínguez 1986, Howe 1990, Willson 1992).

Plants started evolving structures and mechanisms for seed dispersal at least 300 million years ago (Tiffney 1986) and present-day plants accomplish the dispersal of their seeds in many different ways (Stiles 1992, Willson 1992). Dispersal of seeds by fruit-eating animals has received much attention because it is of interest to both plant and animal ecologists. The study of seed dispersal by animals is especially relevant in rainforests, because fruit-eating animals dominate the vertebrate biomass in such ecosystems (Terborgh 1986a) and because more than 75% of tree species in Neotropical forests produce diaspores that seem adapted for animal consumption (Howe 1990).

A crucial aspect in understanding the fruit-frugivore interaction is the fate of seeds after initial deposition by the frugivore (Herrera et al. 1994, Jordano and Herrera 1995, Schupp and Fuentes 1995). Traditionally, this aspect of plant regeneration has received less attention than primary dispersal, mostly because of methodological difficulties and time requirements (Chambers and MacMahon 1994). However, the phase between seed deposition and seedling establishment is likely to have a large effect on the structure of plant communities (Chambers and MacMahon 1994). Also, factors that affect the fate of dispersed seeds determine the degree to which the fitness of a plant depends on primary dispersal by frugivores (Coates-Estrada and Estrada 1988). On the other hand, most studies on seed and seedling survival have focused on factors influencing seed germination and seedling establishment, without taking into consideration the primary dispersal event. Thus, what needs to be more emphasized, is an integration of these two stages, in which the study of seed fate and seedling establishment is linked to characteristics of the initial seed deposition, as determined by the type of primary dispersal.

In the particular case of seeds dispersed through defecation by mammals, many seeds are deposited on the forest floor surrounded by fecal material (Chapman 1989, Julliot 1997, Andresen 1999, McConkey 1999, Chapter 3). The presence of dung around the seeds is likely an important characteristic of these seeds and should be addressed when trying to determine the factors that affect the survival and germination of dispersed seeds. Yet, many mammal-dispersed species can have their seeds dispersed in more than one way, thus, while some seeds may be deposited in mammal defecations, others may not (e.g., they may be dropped, spit out, or regurgitated). Consequently, comparisons of

the fate of seeds dispersed through different means, should include seeds deposited with and without fecal material.

The presence of fecal material can have several potential effects on seed fate and seedling establishment. For example, dung may provide germinating seeds and seedlings with a nutrient boost, or with a water-retaining substrate (Dinerstein and Wemmer 1988, Mittal 1993); it may attract rodents (Janzen 1982b, Estrada and Coates-Estrada 1986, Andresen 1999); and, it may attract dung beetles (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999, Vulinec 1999). In this study I will focus on the effect of dung beetles and rodents attracted to seeds in fecal material.

Both dung beetles and rodents remove seeds from mammal defecations. Dung beetles do not feed on seeds and seed removal occurs incidentally when they bury dung for consumption or oviposition (Halffter and Edmonds 1982). In contrast, rodents actively search for seeds in dung and eat or hoard them for later consumption (Janzen 1982b). It is important to keep in mind, however, that rodents can be important secondary dispersers for some plant species, significantly enhancing the chance of seed survival (Forget 1990, 1991, 1992). Consequently, even though seeds cached by rodents are usually eaten within a few days (Harrington et al. 1997), a few may escape predation and have a higher probability of establishment than seeds not cached by these rodents.

A few studies have shown that dung beetles are able to bury a substantial proportion of seeds present in dung and that this behavior reduces considerably the probability of seed removal by rodents (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999). Thus, it has been suggested that the overall effect of dung beetles on seed fate is positive, through reduction of predation risk. However, it is

still necessary to actually follow the fate of seeds dispersed through defecation by arboreal mammals until germination and seedling establishment, to more accurately assess the overall effect of the dung beetle community on the long-term fate of seeds.

Not only the presence of dung, but also the amount of dung around seeds may affect seed fate and seedling establishment by altering dung beetle and seed predator behavior. Dung amount may be important in several ways. In particular, (i) large dung piles are more likely to attract more and/or larger dung beetles than are small dung piles (Peck and Howden 1984, Chapter 2), (ii) larger dung piles are likely to be found more quickly, due to stronger and more durable olfactory cues for seed predators and dung beetles, and (iii) large dung piles may "hide" seeds from rodent detection (Janzen 1986). The size of dung piles is correlated with the size and behavior of the primary disperser. Thus, through their defecation patterns, different species of primary seed dispersers may also influence the outcome of the interaction between seeds, dung beetles and seed predators (Chapter 3).

Finally, seed size is another factor that may alter dung beetle and seed predator behavior. For example, (i) small seeds may more likely be buried by dung beetles than large seeds (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999, Chapter 2), (ii) larger seeds, having more energy reserves (Foster 1986, Westoby et al. 1992), may be better able to emerge when buried deeply by dung beetles, and (iii) large seeds may emit a stronger olfactory or visual cue for seed predators (Crawley 1992).

The main objective of this study was to link the long-term fate of dispersed seeds and the probability of seedling establishment to the way in which they are deposited by primary dispersers.

The specific objectives were:

1) To determine the effect of presence of dung on long-term seed fate and seedling establishment.
2) To determine the effect of amount of dung on long-term seed fate and seedling establishment.
3) To quantify the depths at which dung beetles naturally bury seeds.
4) To assess if burial depth affects seedling establishment.
5) To examine how seed size affects the relationship between seeds, dung beetles and rodents.

Methods

Study Site and Species

Site. This study was conducted in a 10,000 ha forest reserve in Central Amazonia, between May 1996 and May 1998. The reserve (#1501, also known as "Km 41") is one of several forest reserves that are part of the Biological Dynamics of Forest Fragments Project (BDFFP), which is a joint project of the Brazilian INPA (National Institute for Research in Amazonia) and the US Smithsonian Institution.

The BDFFP reserves are situated about 90 km north of the city of Manaus (2°30'S, 60°W), in the Brazilian state of Amazonas. Mean annual temperature in Manaus is 26.7°C and mean annual rainfall is 2,186 mm (Lovejoy and Bierregaard 1990), with a pronounced dry season from June through October. The soils in this area of the Amazon basin are relatively nutrient poor, sandy or clayey alfic oxisols (latosols; Chauvel 1983) and the forest is classified as tropical moist, according to Holdridge (1967).

The study site is surrounded mostly by primary forest, but some disturbed areas (pasture, secondary forest) occur as little as 10 km away. The reserve is mainly covered by *terra firme* (not subject to river flooding) forest. The canopy is approximately 30-37 m tall, with emergent trees reaching 55 m (Lovejoy and Bierregaard 1990). The flora is very

rich in tree species, with Burseraceae, Sapotaceae, Lecythidaceae and Leguminosae the most important tree families (Rankin-de-Merona et al. 1992).

More detailed descriptions of the BDFFP reserves and background information about the project can be found elsewhere (Lovejoy and Bierregaard 1990, Prance 1990, Rankin-de-Merona et al. 1992, Bierregaard and Stouffer 1997).

Plant species. To assess variability in the role dung beetles may have as secondary seed dispersers of different plant species, I chose to study as many seed species as possible. Plant species differ tremendously in many traits related to seed survival, germination, and seedling establishment (e.g., dormancy, light and water requirements, palatability to seed predators); only by studying several species is one able to reach somewhat more generalizable results.

Information on seed fates of different species also addresses the issue of seed size, at the interspecific level. It is useful to assess the different effects dung beetles may have over a seed-size range that is larger than would be possible to study on an intraspecific level. However, seed species differ in many other characteristics relevant to seed survival and germination, besides seed size. Thus, this approach is not appropriate for assessing the way in which the dung beetle community may interact with intraspecific seed-size variation. The assessment of the effect of intraspecific seed size on the outcome of the seed-dung beetle interaction would allow us to determine if dung beetles are not only playing an ecological role, but may also be playing a role as a selective agent in the evolution of seed traits, in particular seed size. For this purpose I used seeds of two tree species (*Pourouma minor* and *Buchenavia grandis*) that showed large intraspecific seed-size variation (see Table 4.1).

The 15 species of trees that were used in this study (Table 4.1) produce fruits that are either dispersed mostly by mammals or by mammals and birds. All species have relatively large seeds, thus, in the cases in which fruits are eaten by birds, seeds are unlikely to be swallowed and defecated, but rather spit out or regurgitated (Levey 1986, Stiles 1992). Seeds were also classified according to the functional morphology of seedlings. Following Garwood (1996), seedlings were classified by a combination of three dichotomous traits of the cotyledons: exposure, position and texture. With respect to cotyledon exposure, seedlings are phanerocotylar if cotyledons emerge from the seed coat and become totally exposed, and cryptocotylar if the cotyledons remain hidden within the seed coat. In terms of cotyledon position, seedlings are epigeal when cotyledons are raised above ground, and hypogeal if cotyledons are below ground or rest on the surface. Finally, in terms of cotyledon texture, seedlings can have foliaceous cotyledons that become photosynthetic, or fleshy cotyledons that store reserves. Due to constraints in seed and dung availability, not all plant species were used in all three types of experiments described below (seed fate experiment, burial depth experiment, controlled germination experiment; Table 4.1).

Arboreal mammals. Arboreal mammals at the study site likely to disperse the seeds of the focal species by defecation include 6 species of monkeys: *Saguinus midas, Alouatta seniculus, Cebus apella, Ateles paniscus, Pithecia pithecia*, and *Chiropotes satanas.* The latter two act mostly as seed predators. Also, the kinkajou (*Potos flavus*, Procyonidae), the coati (*Nasua nasua* , Procyonidae), the tayra (*Eira barbara*, Mustelidae), and the common opossum (*Didelphis marsupialis*, Didelphidae) are likely to disperse seeds through defecation.

Table 4.1. List of seed species used, plant families, seed dimensions in mm [mean ± 1 SE): length (L), width (W), and thickness (T)], sample size for measurements (N), most likely primary disperser agents (Dis.: M=mammals, B=birds; determined by the fruit/seed size and morphology), seedling functional morphology (Seed.: P = phanerocotylar, C = cryptocotylar, E = epigeal, H = hypogeal, F = foliaceous cotyledons, R = reserve cotyledons; see text for explanation on these terms), and the experiments in which each species was used (Exp.: f = seed fate experiment, d = burial depth experiment, g = controlled germination experiment.

Species	Family	L	W	T	N	Dis.	Seed.	Exp.
Micropholis guyanensis ssp. *Guyanensis*	Sapotaceae	18 ± 0.3	9 ± 0.2	6 ± 0.2	20	M	PEF	f, d, g
Micropholis guyanensis ssp. *Duckeana*	Sapotaceae	14 ± 0.4	8 ± 0.5	5 ± 0.1	10	M	PEF	f
Manilkara bidentata	Sapotaceae	11 ± 0.2	6 ± 0.1	3 ± 0.1	20	M	PEF	f
Pouteria durlandii	Sapotaceae	27 ± 0.7	11 ± 0.1	9 ± 0.2	10	M	PER	f, d
Pouteria sp. 18	Sapotaceae	20 ± 0.3	10 ± 0.1	6 ± 0.1	10	M	PER	f, g
Pouteria sp. 7	Sapotaceae	20 ± 0.3	12 ± 0.5	12 ± 0.7	10	M	PER	g
Pourouma guianensis	Moraceae	11 ± 0.8	7 ± 0.3	6 ± 0.3	10	M, B	CHR	f, d, g
Pourouma minor	Moraceae					M, B	CHR	f, d, g
Large seeds		18 ± 0.2	10 ± 0.1	7 ± 0.1	10			
Small seeds		12 ± 0.2	9 ± 0.1	5 ± 0.1	10			
Helicostylis tomentosa	Moraceae	11 ± 0.2	8 ± 0.1	6 ± 0.2	10	M, B	CHR	f, d, g
Helicostylis scabra	Moraceae	5 ± 0.2	5 ± 0.2	4 ± 0.1	10	M, B	?	d
Minquartia guianensis	Olacaceae	22 ± 0.5	12 ± 0.6	12 ± 0.6	10	M	PER	f, g
Buchenavia grandi	Combretaceae					M	PEF	f, d, g
Large seeds		30 ± 0.4	13 ± 0.3	13 ± 0.3	10			
Small seeds		21 ± 0.3	10 ± 0.1	10 ± 0.1	10			
Ocotea percurrens	Lauraceae	12 ± 0.2	8 ± 0.1	7 ± 0.1	10	M, B	CHR	f, g
Byrsonima crispa	Malpighiaceae	8 ± 0.2	8 ± 0.2	8 ± 0.2	15	M, B	?	d
Quiinaceae sp.	Quiinaceae	9 ± 0.2	7 ± 0.1	5 ± 0.2	10	M, B	PEF	g

Because I needed large amounts of fecal material for conducting the experiments described below, I used the dung of howler monkeys (*Alouatta seniculus*) in all experiments. Howler monkeys are abundant in the study site, groups are located readily and the collection of large quantities of dung is feasible. Although howler monkeys are generally considered to be the most folivorous of Neotropical primates, they also

consume large amounts of fruit whenever it is available, and disperse the viable seeds of many plant species (Neves and Rylands 1991, Gilbert 1994, Julliot 1996a, b, Chapter 3).

Dung beetles. The dung beetle (Coleoptera: Scarabaeidae) community in the study area is made up of approximately 60-65 species (Klein 1989, Chapters 2 and 5). Many are very abundant year-round and most mammal dung disappears within a few hours, due to their dung burying and relocation behavior. Dung beetle species attracted to howler monkey dung vary in size from a few millimeters to about 40 mm. See Chapter 2 for a detailed description of the dung beetle community at the study site and the beetles' behavior as secondary dispersers.

Seed Fate Experiments: Effects of Dung Presence, Dung amount, and Seed size

To assess how the regeneration of plants is affected by the dung beetles I followed the fate of 2537 seeds belonging to 11 species (Table 4.2). For all species I assessed the effect of the presence of dung, by comparing seed fate for seeds with no dung and seeds surrounded by 10 g of dung. For 6 of the seed species I also used more than one dung treatment (5 g and/or 25 g of dung in addition to the 10 g treatment) to assess the effect of dung amount on seed fate. The different amounts of dung used may represent a range of dung-pile sizes produced by one species of frugivore (e.g., Chapter 3), or average dung-pile sizes produced by different species of mammals (e.g., Estrada et al. 1993).

Finally, for two of the species, *B. grandis* and *P. minor*, I used large and small seeds, to determine if dung beetles, through their seed-burying behavior, may provide selective pressure in the evolution of seed size (these two species were chosen because they showed pronounced intraspecific seed-size variation and enough seeds could be found for each extreme size category).

Table 4.2 Species used in seed fate experiments, with the times at which experiments with each species were started and intervals at which seeds were checked, and number of seeds used in each treatment. All species were used to assess the effect of presence of dung; * indicates species used to assess the effect of amount of dung, and @ indicates species used to address the effect of intraspecific seed size variation.

Species	Month and year when experiment was started	Checking intervals (in weeks, except 1d, which is one day after seeds were set out)	Treatments (in grams of dung per seed / Number of seeds in each treatment)
Micropholis guyanensis ssp. *Guyanensis**	Jan-Feb 97	1d, 2, 4, 6, 8, 10, 12, 14, 16, 26	0 g/ 160; 5 g/ 160; 10 g/ 160; 25 g/ 160
Micropholis guyanensis ssp. *Duckeana*	Feb 97	1d, 2, 4, 8, 12	0 g/ 70; 10 g/ 70
*Manilkara bidentata**	Feb 97	1d, 2, 4, 6, 8, 10, 12, 14, 16, 27, 32, 38, 50	0 g/ 70; 10 g/ 70
Pouteria durlandii	Feb 98	1d, 2, 4, 8, 12	0 g/ 108; 10 g/ 108
Pouteria sp. 18	Feb 98	1d, 2, 4, 7	0 g/ 50; 10 g/ 50
Pourouma guianensis	Mar 98	1d, 4, 6, 8	0 g/ 108; 10 g/ 108
*Pourouma minor** @ Large seeds Small seeds	Mar 97	1d, 2, 4, 6, 8, 11	0 g/ 70; 5 g/ 70; 10 g/70 10 g/ 60
*Helicostylis tomentosa**	May 97	1d, 2, 4, 6, 8, 10, 12	0 g/ 30; 5 g/ 30; 10 g/ 30
Minquartia guianensis	Aug 97	1d, 5, 7, 9, 11, 15, 19, 21, 23, 28, 30, 32, 35, 40	0 g/ 35; 10 g/ 50
*Buchenavia grandis** @ Large seeds Small seeds	Apr 97	1d, 2, 4, 6, 8, 10, 12, 14	0 g/ 50; 10 g/ 50; 25 g/ 50 0 g/ 50; 10 g/ 50; 25 g/ 50
*Ocotea percurrens**	Sep-Oct 97	1d, 2, 4, 6 10, 14, 16, 18, 20, 23, 25, 28, 33	0 g/ 49; 5 g/ 100; 10 g/ 100

I collected seeds mostly from howler monkey defecations, but I also used seeds from fruits collected under trees and from tree crowns. I used only one seed source for each species, except for *M. g.* spp. *guyanensis* and *B. grandis*, for which I used seeds from defecation and seeds from fruits (Chi-square tests on frequencies of germinated seeds for defecated and tree seeds: all P's > 0.05). Studying the effect of passage through

the gut on germination success of seeds dispersed by red howler monkeys in French Guiana, Julliot (1996b) found that most plant species were not affected by passage through the gut. Also, for these species, seeds of each source were represented equally among the treatments being compared.

I washed seeds and put them in the shade. Seeds were used within a few days after collection to avoid desiccation, which has been shown to significantly affect germination probabilities for some species (Benitez-Malvido 1995). Seeds that had to be stored for longer periods were stored in plastic bags in an insulated box with ice (this treatment did not affect germination, Chi-square tests: all P's > 0.05).

Dung was usually collected in the morning (0700-1000 h). Seeds > 3 mm were removed to avoid their interference with experimental seeds. Dung was kept in plastic bags in the shade. Since the freshness of dung is very important for its attractiveness to dung beetles (Howden and Nealis 1975, Halffter and Edmonds 1982), the dung was generally used for experiments on the same day. When the dung was used the next day, it was kept in an insulated box with ice. The dung was never stored for more than 36 h.

To facilitate finding seeds that were moved by dung beetles or rodents, all seeds used in this experiment were marked with 50 cm of strong white nylon thread. The thread was attached to the seed using an epoxy cement that dried within 2 h. Care was taken not to apply the cement to any part of the seed that might interfere with germination (e.g., the scar of Sapotaceae seeds). Seeds were placed on the forest floor, along transects, one seed every 10 m. Distances of 5 m have been considered sufficient to assure independence in seed removal/predation experiments in rainforests (Burkey 1993, Blate et al. 1998). Seeds were placed at locations where the leaf litter had been cleared in a small area (10 x 10

cm) and a 20 cm long wooden stick with a numbered piece of flagging tape had been placed the previous day.

Treatments included single seeds with no dung and single seeds surrounded by dung. To assess the effect of presence of dung, 10 g dung piles were used, and to assess the effect of amount of dung 5 g and/or 25 g dung piles were used besides the 10 g dung piles. The amounts of dung were chosen according to the natural weight of howler monkey fecal piles: 48% of all dung in 13 defecation samples (4772 g of dung; using the term defecation sample as defined in Chapter 3) was made up of individual dung piles that weighed 5-30 g (Chapter 3). The no-dung treatment simulates seeds that have been dispersed in other ways (e.g., dropped, spit out). Table 4.2 shows the treatments used for each of the seed species and the number of seeds used in each treatment. Because both dung and seed availiability were limited for some species and during some periods of the year, not all dung treatments could be used with all seed species.

Transects varied in length, depending on the number of treatments used for each species and the amount of seeds and dung available on a given day. The different treatments for each species were set out simultaneously, alternating them along the transect. For some species, additional transects included an incomplete set of treatments to increase the sample size of one or two of the treatments (e.g., the 10g treatment for *M. bidentata*, *P. durlandii* and *M. guianensis*, and the 5g and 10g treatments for *O. percurrens*; Table 4.2). Between 20 and 120 seeds (with and without dung) were set out on any single day. All transects were set out in the afternoon, between 1500 h and 1800 h. This was done to avoid having time of day as a confounding factor, since the diurnal and

nocturnal dung beetle assemblages are usually different (Hanski and Cambefort 1991c, Chapter 2).

I checked transects after 1 day and then periodically until predation/removal, or seedling establishment. The number of checks and time intervals between checks are shown in Table 4.2. For each seed I recorded its fate (seed alive, seed preyed/removed, seed germinating, seedling) and location (buried or on the surface and horizontal distance measured in increments of 5 cm). A seedling was considered established when it had two leaves (either true leaves or, in the case of *Micropholis* spp., *M. bidentata*, *B. grandis*, and Quiinaceae sp., leaf-like cotyledons). When seeds had been removed, an effort was made to find the seed and/or thread; an area of approximately 5 m around the location was searched. Of the seeds removed by rodents and found by me, all had either been eaten (empty husk was found) or hidden in a place unfavorable for seedling establishment (e.g., inside hollow logs). Thus, seeds that were not found were assumed to be not viable or dead. This might result in an overestimation of seed death caused due to rodent activity if rodents cache seeds and if some cached seeds survived. At the end of the experimental period for each species, the buried seeds that had not emerged as seedlings were unburied to determine whether the seed had not germinated, whether it had been preyed upon after germination, or whether the emerging seedling had not been able to reach the surface.

Burial Depth Experiment

Since in the seed fate experiments I did not unbury the seeds buried by dung beetles (except those that did not emerge as seedlings), I could not determine the depths at which most seeds had been buried by dung beetles. Thus, to quantify burial depths I conducted a separate experiment in which I placed individual seeds surrounded by 10 g of dung every

10 m along transects, in a way similar to the seed fate experiment. In this experiment, however, I unburied the seeds after two days, and measured the depths of burial to the nearest centimeter. Due to the difficulty of finding large quantities of dung during some times of the study period (e.g., during the rainy season the monkeys were more difficult to locate), I was able to conduct this experiment with only six of the species used in the seed fate experiment (*M.* ssp. *guyanensis*, *B. grandis*, *P. guianensis*, *P. minor*, *P. durlandii* and *H. tomentosa*) and two additional species for which seed fate data are not available (*H. scabra* and *B. crispa*). For each species, the number of seeds naturally buried by dung beetles and for which burial depth was recorded are given in Table 4.3. Horizontal movement of the seeds, to the nearest 5 cm, was also recorded. Seeds in this experiment were re-used, but they were not used in the seed-fate experiment. To test whether dung amount also had an effect on burial depth, I used different amounts of dung for *P. minor*, *B. grandis*, *H. scabra*, and *B. crispa* (Table 4.3). Finally, to test whether intraspecific seed size variation in *P. minor* and *B. grandis* had an effect on burial depth, I performed this experiment for both large and small seeds of these two species.

Controlled Germination Experiment

To assess the effect of burial depth on seedling emergence and establishment, seeds of eight of the species used in seed fate experiments and two additional species for which seed fate data are not available (Quiinaceae sp. and *Pouteria* sp. 7) were placed individually in plastic cups with forest soil. Treatments were: seeds on top of the soil and seeds buried at different depths: 1 cm, 5 cm and 10 cm. After experiments with the first six species, it became clear for some species that an abrupt drop in the percentage of seedlings emerging occurred between burial depths of 1 and 5 cm. Therefore, for the

remaining four species, an additional treatment of 3 cm depth was added. The depths

used are within the observed range of depths at which seeds are naturally buried by dung

beetles (see Fig. 4.4).

Table 4.3 Species and dung treatments used in burial-depth experiment. N indicates the number of seeds that were buried naturally by dung beetles and subsequently found and for which the burial depth was measured.

Species		Dung treatment	N
Micropholis guyanensis ssp. *guyanensis*		10 g	51
Pouteria durlandii		10 g	52
Pourouma guianensis		10g	56
Pourouma minor	Large seeds	5 g	27
	Large seeds	10 g	23
	Small seeds	10 g	28
Helicostylis tomentosa		5 g	7
		10 g	17
Helicostylis scabra		10 g	39
Buchenavia grandis	Large seeds	10 g	10
	Large seeds	25 g	25
	Small seeds	10 g	16
	Small seeds	25 g	25
Byrsonima crispa		5 g	46
		10 g	68
		25 g	43

Between 15 and 60 seeds were used per species per depth treatment. The plastic

cups had drainage holes and were placed on the forest floor in an area where the

understory and midstory vegetation had been cleared, but the canopy was intact. A plastic

mosquito net was placed around and on top of groups of cups, to prevent seed and

seedling predation. The number of seedlings establishing in each treatment was recorded.

The duration of this experiment varied according to the time until germination and

seedling emergence for each species and burial depth (between 10 days and 7 months).

Data Analysis

Since most of the data are frequencies of discrete variables, Chi-square tests were used for analyses. When data in 2 x 2 contingency tables contained more than 20% of cells with frequencies < 5, the Fisher Exact test was used. When 2 x R tables had to be partitioned after the main test, the procedure recommended by Siegel and Catellan (1988), in which independent subtables are created was used. Alternatively, non-orthogonal pair-wise comparisons were performed using a corrected critical value (Sokal and Rohlf 1995).

Seed length was found to be highly correlated with both seed width (Pearson correlation coefficient, r = 0.91) and seed thickness (r = 0.73). Thus, only seed length was used as the measure of seed size in analyses. Also, from the dung beetles' perspective, seed length is probably what matters more in determining whether they remove or do not remove a seed from the dung portion they relocate. Pearson correlation coefficients were used to test for correlation between seed size and percentage of seeds buried, depth of burial, percentage of seeds moved horizontally, and horizontal distance moved.

A linear regression was used to further analyze the relationship between seed size and percentage of seeds buried by dung beetles. A multiple regression procedure was used to fit a curvilinear regression to the relationship between seed size and burial depth (independent variables: seed length and (seed length)^2; Sokal and Rohlf 1995). Two linear regressions were also used in the controlled germination experiment to test for a relationship between seed size and the ability of a seed to emerge from depths of 5 and 10 cm. The dependent variables were the percentage of seedlings establishing from seeds at 5 and 10 cm, relative to the percentage of seedlings establishing at 1 cm, i.e.,

establishment at 1 cm was used as the baseline establishment potential for buried seeds. For *O. percurrens*, the baseline used was the establishment at 0 cm, because seeds of this species germinated poorly when covered by soil, regardless of depth. An angular transformation and a logarithmic transformation were performed on the dependent and independent (seed length) variables, respectively.

Wilcoxon signed ranks tests were used to test the following effects: burial condition (whether buried by beetles or not) on seedling emergence, dung presence on predation of seeds not buried by beetles, dung amount on burial condition, and dung amount on burial depth. For these tests, all species were considered together, using each species as an independent paired sample. For species used with three dung-amount treatments, orthogonal combinations were used as independent samples, i.e., *M. g.* spp. *guyanensis* and *B. cripsa* were used as two samples each: 5g of dung vs. 10g of dung, and 10g of dung vs. 25g of dung).

To analyze individual species for the effect of the amount of dung and seed size, on burial depth, Kruskall-Wallis and Mann-Whitney tests were used.

In the case of three-way frequency tables, log-linear models were fit to test for significance in interaction among main factors. In each case, several models were tested, starting with the most complex, and dropping the least significant terms in sequence. In the results section I report the outcome of the most parsimonious model, i.e., the model with the least number of main factors and interaction terms able to fit the data. Likelihood-ratio Chi-square tests were used to test the fit of each model; a non-significant Chi-square value ($P > 0.05$) indicates an adequate fit of a model (Sokal and Rohlf 1995).

The log-linear procedure was used to test for interactions among the following main

factors:

- Seedling emergence, seed size, and burial condition (whether buried by dung beetles or not); for *B. grandis* and *P. minor.*
- Seedling emergence, amount of dung, and burial condition; for all species used with more than one dung-amount treatment.
- Depth of burial, seed size, and amount of dung; for *B. grandis.*
- Burial condition, seed size, and amount of dung; for *B. grandis.*

All analyses were performed using Systat® 6.0 (SPSS Inc. 1996).

Results

Seed Fate Experiment

The fate of dispersed seeds of all plant species is shown if Fig. 4.1. In this figure,

seeds have not been grouped just in terms of their initial treatment (no dung, 5 g, 10 g, 25

g of dung), but in terms of both their initial treatment and whether or not they were buried

by dung beetles. Thus, Fig. 4.1 shows the fate of: (i) seeds placed on the forest floor

without dung and consequently lying on the soil surface (hereafter called "no-dung

seeds"), (ii) seeds initially placed with dung (5 g, 10 g, or 25 g) but that were not buried

by dung beetles during dung removal (hereafter called "dung-surface seeds"), and (iii)

seeds initially placed with dung and buried by dung beetles during dung removal

(hereafter called "dung-buried seeds"). Dung-surface seeds together with dung-buried

seeds constitute all the seeds originally placed with dung (hereafter called "dung seeds").

The results of the seed fate experiment are divided in three sections. In the first I

report the results of the comparisons of seeds without dung and seeds with 10 g of dung

to determine the effect of presence of dung on seed fate. In the second section I assess

the effect of amount of dung on seed fate. Finally, in the third section I analyze the seed
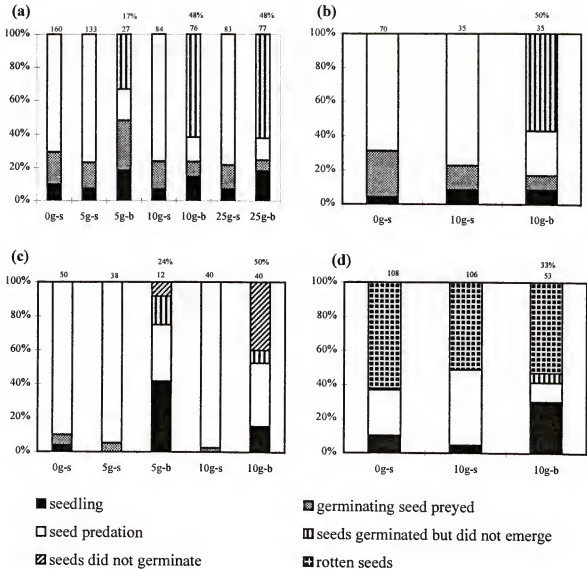
Figure 4.1. Fate of seeds placed with no dung on the surface (0g-s = no-dung seeds), placed with 5, 10 and/or 25 g of dung on the surface, and subsequently buried by beetles (dung-buried seeds: 5g-b, 10g-b, or 25g-b), and of seeds placed with dung and not buried by beetles (dung-surface seeds: 5g-s, 10g-s, 25g-s). (a) *Micropholis guyanensis* ssp. *guyanensis*, (b) *Micropholis guyanensis* ssp. *duckeana*, (c) *Manilkara bidentata*, (d) *Pouteria durlandii*, (e) *Pouteria* sp. 18, (f) *Pourouma guianensis*, (g) *Pourouma minor* (large and small seeds), (h) *Helicostylis tomentosa*, (i) *Minquartia guianensis*, (j) *Buchenavia grandis*, large seeds, (k) *Buchenavia grandis* small seeds, (l) *Ocotea percurrens*. Numbers on top of each bar indicate number of seeds; for dung-buried seeds, the percentage of seeds buried by dung beetles is also given.

Figure 4.1 -- continued

Figure 4.1 -- continued

fate data for the two seed species for which the seed fate experiments were conducted
with large and small seeds to test for an effect of intraspecific seed size variation.

   a) Effect of presence of dung. Twenty-six to 67 percent of the seeds surrounded by
10g of dung were buried by dung beetles, depending on the species. More than 50% of
the variation in percent of seeds buried was explained by seed size, with smaller seeds
buried more often than larger seeds ($F_{1,13} = 13.88$, $P = 0.003$, $r^2 = 0.52$; Fig. 4.2). The
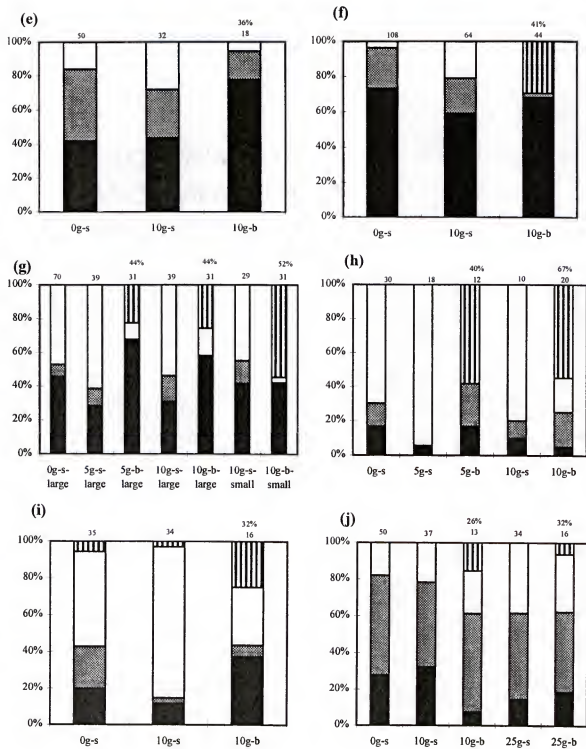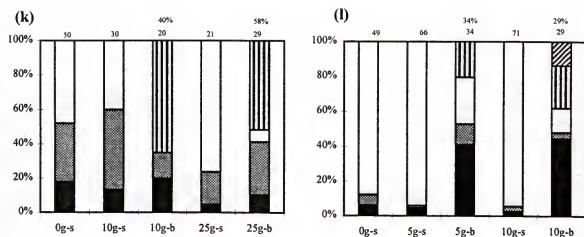depth of burial varied between 0.5 cm and 20 cm (see below for more details on burial
depths). Dung beetles not only moved seeds vertically, but also horizontally. Horizontal
movement of a seed could occur regardless of whether the seed was buried by beetles.
Mean percentage of seeds moved horizontally varied between 5% and 36% for the
different species and mean horizontal distances varied between 10 and 33 cm. Neither the
percentage of seeds moved horizontally, nor the distance moved, was correlated with
seed size ($r = -0.343$, $P = 0.275$; and, $r = 0.363$, $P = 0.247$, respectively).

   Analyzing all species together, i.e., considering each species as a sampling unit, the
presence of dung did not have an effect on the percentage of seedlings establishing.
(Wilcoxon signed ranks test, $T^+ = 31$, $N = 10$, $P = 0.38$). Similarly, when each of the
species was analyzed separately, it was found that for all of them, except *P. guianensis*,
the same proportion of seedlings emerged from no-dung seeds than from dung seeds with
10 g of dung (for all Fisher exact tests and Chi-square tests: P's > 0.05; Table 4.4). For *P.
guianensis*, more seedlings emerged from no-dung seeds than from dung-seeds ($\chi^2 = 8.8$,
d.f. = 1, P < 0.01).

   When comparing no-dung seeds, dung-surface seeds and dung-buried seeds, five of
the eleven species showed significant differences in the number of seedlings emerging

Table 4.4. Percentage of seedlings establishing from no-dung seeds (A), dung seeds (regardless of whether buried by dung beetles or not) (B), dung-surface seeds (C) and dung-buried seeds (D). N is the total number of seeds used, i.e. seeds in A + seeds in B; number of seeds in B = seeds in C + seeds in D. Also given are the results of statistical analyses comparing the frequencies of established seedlings. When chi-square tests were performed, the value of the test statistic is given (X^2), when Fisher exact tests were performed the probability value is given (P). The last three columns represent post-hoc pairwise comparisons, with exact corrected probability values for non-orthogonal post-hoc tests (Sokal and Rohlf 1995). n.s. = P > 0.05, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Species	N	% seedlings establishing from:				A vs C vs D	A vs B	A vs C	A vs D	C vs D
		A no dung	B dung	C dung surface	D dung buried					
M.g.guyanensis [1]	320	10.0	10.6	7.1	14.5	X^2=2.31, n.s.	X^2=0.03, n.s.	X^2=0.78, n.s.	X^2=3.21, n.s.	X^2=10.15, **
M.g.guyanensis [2]	640	10.0	10.8	7.3	16.7	X^2=10.41, **	X^2=0.87, n.s.	P=0.39, n.s.	P=0.39, n.s.	P=1, n.s.
M.g.duckeana [1]	140	4.3	8.6	8.6	8.6		P=0.49, n.s.	P=0.5, n.s.	P=0.13, n.s.	P=0.39, n.s.
M.bidentata [1]	130	4.0	7.5	0.0	15.0		P=0.71, n.s.	P=0.15, n.s.	P=0.02, *	P=0.03, *
M.bidentata [2]	180	4.0	8.5	0.0	21.2	X^2=22.29, ***	P=0.52, n.s.	X^2=2.31, n.s.	X^2=10.19, **	P=0.001, **
P.durlandii [1]	267	10.2	13.2	4.7	30.2	X^2=7.29, *	X^2=0.56, n.s.	X^2=0.02, n.s.	X^2=6.78, *	X^2=20.00, **
P. sp.18 [1]	100	42.0	56.0	43.8	77.8	X^2=15.51, ***	X^2=1.96, n.s.	X^2=14.77, **	X^2=0.38, n.s.	X^2=5.41, n.s.
P.guianensis [1]	216	73.1	53.8	43.8	68.2	X^2=3.07, n.s.	X^2=8.80, **			X^2=6.25, *
P.minor [1,3]	200	45.7	42.3	35.3	50.0	X^2=10.97, *	X^2=0.21, n.s.	X^2=3.04, n.s.	X^2=1.66, n.s.	
P.minor [2,3]	270	45.7	43.5	32.7	55.9		X^2=0.10, n.s.		P=0.38, n.s.	X^2=10.90, **
H.tomentosa [1]	60	16.7	6.7	10.0	5.3		P=0.42, n.s.	P=1, n.s.	P=0.47, n.s.	P=1, n.s.
H.tomentosa [1]	90	16.7	8.3	7.1	9.4		P=0.29, n.s.	P=0.42, n.s.		P=1, n.s.
M.guianensis [1]	85	20.0	21.0	11.8	37.5	X^2=4.5, n.s.	X^2=0.00, n.s.			
B.grandis [1,3]	200	23.0	21.0	23.9	15.2	X^2=1.09, n.s.	X^2=0.12, n.s.			
B.grandis [2,3]	300	23.0	16.5	18.0	14.1	X^2=2.33, n.s.	X^2=1.85, n.s.			
O.percurrens [1]	149	6.1	15.0	2.8	44.8	X^2=36.65, ***	X^2=2.44, n.s.	X^2=0.79, n.s.	X^2=16.74, **	X^2=28.50, ***
O.percurrens [2]	249	6.1	16.0	3.6	42.9	X^2=58.09, ***	X^2=3.17, n.s.	P=0.44, n.s.	X^2=18.96, ***	X^2=49.36, ***

[1] seeds with dung are only from the 10 g treatment

[2] seeds with dung are from all dung treatments used for that species pooled together

[3] includes large and small seeds
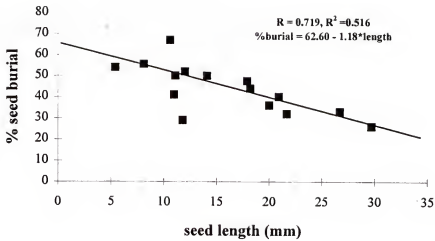
Figure 4.2. Relationship between seed size and percentage of seeds surrounded by 10g of dung, buried by dung beetles. Data come from the 11 species used in seed fate experiment (see Table 4.3), using small and large seeds from *B. grandis* and *P. minor* separately, and also from two species used only in the burial-depth experiment (*B. crispa* and *H. scabra*).
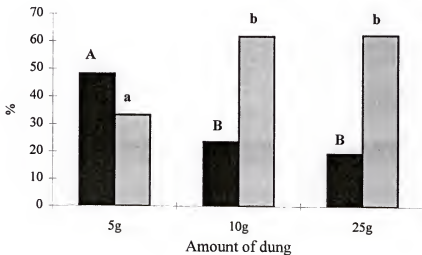
Figure 4.3. Percentage of seed and germinating seed predation (black bars), and percentage of seeds not emerging (grey bars), for *Micropholis guyanensis* seeds surrounded by different amounts of dung and buried by dung beetles. N = 27 for 5 g , N = 76 for 10 g, and N =77 for 25 g. Matching letters above bars identify statistically homogeneous samples (P>0.05; upper-case letters for seed predation and lower-case letters for non-emergence).

(see Table 4.4 for results of statistical analyses). To increase sample size, I also repeated the analysis pooling dung treatments (5 g, 10 g and 25 g) for those species for which more than one dung treatment was used. By doing this, two additional species showed significant differences in the number of seedlings establishing from no-dung seeds, dung-surface seeds, and dung-buried seeds (Table 4.4). Six of the seven species with significant results, showed no difference in seedling establishment between no-dung seeds and dung-surface seeds. When dung-surface seeds were compared to dung-buried seeds, these six species showed a significantly higher percentage of seedlings emerging from dung-buried seeds (Table 4.4). Four species also showed a significantly lower percentage of seedlings emerging from no-dung seeds that from dung-buried seeds. In the case of *P. guianensis*, the same percentage of seedlings emerged from no-dung seeds and dung-buried seeds, and these percentages were significantly higher than the percentage of seedlings emerging from dung-surface seeds.

Considering only the seeds surrounded by 10 g of fecal material and analyzing all species together (i.e., each species constitutes a sampling unit) for seedling emergence, more seedlings established when seeds were buried by dung beetles (33%), than when seeds remained on the surface (17%; Wilcoxon signed ranks test, $T^+ = 51$, $N = 10$, $P = 0.007$).

Even though no statistical differences were found for individual species in the percentage of seedlings emerging from no-dung seeds and dung-surface seeds, when all species were analyzed together, 10 g dung-surface seeds suffered higher predation than no-dung seeds (Fig. 4.1; Wilcoxon signed ranks test, $T^+ = 64$, $N = 11$, $P = 0.0015$). When preyed seeds were pooled with preyed germinating seeds, the difference was still

significant, but at a lower level (Wilcoxon signed ranks test, $T^+ = 53$, N = 11, P = 0.04), suggesting that the effect diminishes with time.

Of seeds placed with 10 g of dung and buried by dung beetles, 0-62% were either not able to emerge after they had germinated or did not germinate (Fig. 4.1). The latter occurred only for two species (*M. bidentata* and *O. percurrens*). I did not test for viability of these seeds. However, the results for these species are conservative, since for both species a significantly higher percentage of seedlings emerged from buried seeds than from surface seeds. Thus, if the seeds that did not germinate during the study period were viable and eventually germinated, this would only make the observed difference more significant. For *M. guianensis*, 6% of no-dung seeds and 3% of dung-surface seeds also were not able to establish as seedlings because the radicle of the germinating seed could not penetrate the ground and the cotyledons remained covered by the seed husk and eventually desiccated.

b) Effect of dung amount. Considering all species together and analyzing seeds placed with dung, more seeds were buried when they were surrounded by larger amounts of dung than by smaller amounts (Wilcoxon signed ranks test, $T^+ = 43$, N = 9, P = 0.0059). All species except two showed this trend. For *O. percurrens* more seeds were buried in the 5 g than in the 10 g treatment, but the difference was not significant ($\chi^2 = 0.58$, d.f. = 1, P = 0.45), and for *P. minor*, the same number of seeds was buried in the 5g and 10g treatments ($\chi^2 = 0$, d.f. = 1, P = 1; Fig. 4.1). A statistically significant higher percentage of seeds buried by dung beetles for seeds with more dung vs. less dung was found in *M.* ssp. *guyanensis* ($\chi^2 = 4.57$, d.f. =2, P < 0.001), *B. crispa* ($\chi^2 = 23.50$, d.f. = 2, P < 0.001 for *B.c.*), *M. bidentata* ($\chi^2 = 8.67$, d.f. =1, P < 0.01), and *H. tomentosa* ($\chi^2 =$

4.29, d.f. = 1, P < 0.05). In the case of small *B. grandis* seeds the test was suggestive of significance (χ^2 = 3.24, d.f. = 1, P = 0.07) and for large *B. grandis* seeds the same proportion of seeds was buried with 10 g as with 25 g. For *B. grandis* a log-linear model was fit to test for interactions between burial condition (whether buried by dung beetles or not), seed size and amount of dung. In the most parsimonious model (likelihood ratio χ^2 = 3.70, P = 0.45) only significant interaction of the main factors was between burial condition and seed size, which means that whether or not a seed is buried by beetles will depend on seed size.

To see if significant interactions existed between three main factors: seedling establishment, amount of dung and burial condition, a log-linear model was fit for each species, for seeds placed with dung. For *H. tomentosa*, *O. percurrens* and *B. grandis* (large and small seeds analyzed separately) no significant interactions were found and the main factors were therefore considered independent. For *P. minor*, *M.* ssp. *guyanensis* and *M. bidentata*, seedling establishment interacted with burial condition, which means that whether or not a seedling establishes depends on whether or not the seed had been buried by beetles. For the latter two species the interaction between dung amount and burial condition was also significant, indicating that whether or not a seed is buried will depend on the amount of dung surrounding the seed.

Considering only the seeds with dung that were buried by dung beetles, species differed in the effect that amount of dung had on the number of seedlings emerging, the number of seeds preyed upon (pooling seeds and germinating seeds), and the number of seeds that did not emerge (Fig 4.1). None of the species, except *M. bidentata*, displayed differences in the number of seedlings emerging from buried seeds with more than with

less dung (Chi-square tests and Fisher exact tests, all P's ≥ 0.1). In the case of *M. bidentata,* significantly more seedlings emerged from 5 g dung-buried seeds (42%) than from 10g dung-buried seeds (15%; $\chi^2 = 3.93$, d.f. $= 1$, P < 0.05). Regarding the number of seeds preyed upon and the number of seeds that did not emerge, only for *M. g.* ssp. *guyanensis* were observed frequencies large enough to detect statistical differences. For this species, seed predation decreased with larger amounts of dung ($\chi^2 = 8.07$, d.f. $= 2$, P < 0.05; Chochran's test of linear trend $= 6.78$, P < 0.01), but the number of seeds not emerging was higher for the 10 and 25 g treatments than for the 5g treatment ($\chi^2 = 7.73$, d.f. $= 2$, P < 0.05; Fig. 4.3).

c) Effect of intraspecific variation in seed size. For *P. minor* seeds, 44% of 'large' seeds surrounded by 10g of dung were buried by dung beetles, whereas 52% of 'small' seeds were buried, but this difference was not statistically significant ($\chi^2 = 0.71$, d.f. $= 1$, P $= 0.4$). A higher percentage of seedlings emerged from 'small' seeds that remained on the surface than from 'large' seeds on the surface. A higher percentage of seedlings emerged from 'large' buried seeds, than from 'small' buried seeds, due to a large proportion (55%) of the 'small' buried seeds not being able to emerge after germination (Fig. 4.1). However, these differences were not statistically significant (Fisher exact tests, P's > 0.05). This lack of statistically significant interactions between seed size, burial condition, and percentage of seedlings, was confirmed by fitting a log-linear model. The simplest model (i.e., the model with no interaction terms) had an adequate fit (likelihood ratio $\chi^2 = 6.023$, P $= 0.197$), which means that the factors are independent.

For *B. grandis* seeds, a lower percentage (29%) of 'large' seeds with dung (10g and 25g treatments pooled) were buried by dung beetles than were 'small' seeds (49%). For

this species the difference was statistically significant ($\chi^2 = 8.41$, d.f. =1, P < 0.01). No differences were found in the percentages of seedlings emerging from large vs. small seeds, both for no-dung seeds and for dung-buried seeds (Chi-square tests, P > 0.1). When analyzing dung-surface seeds, a significantly higher percentage of seedlings emerged from large than from small seeds ($\chi^2 = 4.01$, d.f. = 1, P < 0.05). The simplest log-linear model to fit the data (likelihood ratio $\chi^2 = 8.04$, P = 0.1541), included a single significant interaction term, the one between seed size and burial condition, which means that whether a seed is buried or not depends on seed size.

Small *B. grandis* seeds were buried significantly more deeply than large seeds; for *P. minor* this trend was also observed, but the difference in burial depth for small vs. large seeds was not significant (see next section for statistical tests).

Burial Depth Experiment

The highest percentage of seeds was buried between 0.5 and 2 cm for all species, but many seeds were buried at greater depths (Fig. 4.4). Seeds were found to a maximum depth of 20cm. Median depths ranged from 0.5 to 8 cm among species. A significant curvilinear relationship was found between mean burial depth and seed size: large seeds (> 25 mm in length) and small seeds (< 10 mm) were buried less deeply, while medium-sized seeds (10 – 25 mm) were buried more deeply ($F_{2,7} = 4.90$, P = 0.047, r = 0.764; Fig. 4.5).

When the effect of amount of dung on burial depth was analyzed, taking each species as a sampling unit, the median burial depth was higher for seeds surrounded by more dung than by less dung (Wilcoxon signed ranks test, $T^+ = 20$, N = 6, P = 0.03). All species, except *B. grandis*, showed this trend. When performing statistical tests on
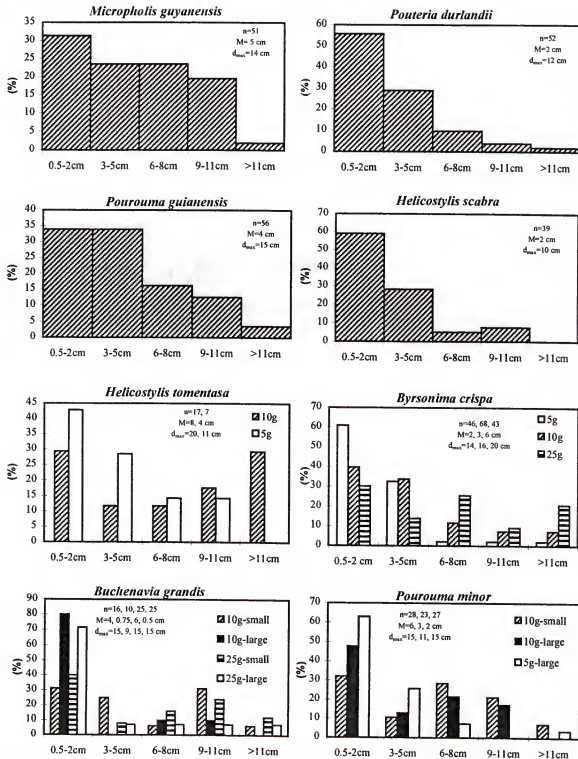
Figure 4.4. Percentage of seeds buried at different depth intervals. For species with one dung treatment, 10g of dung were used; for the others the amount of dung in shown in the legends. For *Pourouma minor* and *Buchenavia grandis* large and small seeds are compared. Sample sizes, median burial depths (M), and maximum burial depth (d_{max}) are also shown (in the same order as in the legend, for species with more than one size and/or dung treatments).

R = 0.764, R2 = 0.583
depth = 0.969 + 0.614*length - 0.02*length^2
F = 4.895, p =0.047
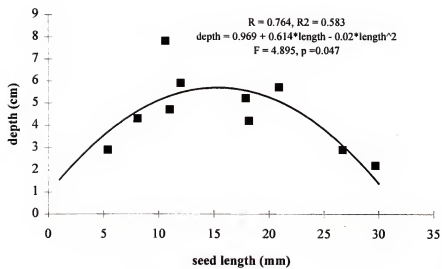
Figure 4.5. Relationship between seed length and mean burial depth.

individual species, the variation in burial depth was too large to detect significant differences in any of the species (Mann-Whitney tests, $P > 0.05$), except *B. crispa*. In the case of *B. crispa* the result of larger burial depth with larger amounts of dung was significant with all dung treatments (Kruskall-Wallis statistic = 18.687, $P < 0.001$; multiple comparisons with adjusted critical value: 5 g vs. 10g, $P < 0.05$, 10 g vs. 25 g, $P < 0.05$, 5 g vs. 25 g, $P < 0.05$).

Intraspecific seed size significantly affected the depth of seed burial for *B. grandis*, with larger seeds being buried less deeply than smaller seeds (Mann-Whitney tests, for seeds with 10 g of dung: $U = 122$, $N = 16$ for small and 10 for large seeds, $P = 0.024$; for seeds with 25 g of dung: $U = 251$, $N = 25$ for both small and large seeds, $P = 0.022$), but not for *P. minor* ($U = 401.5$, $N = 28$ for small and 23 for large seeds, $P = 0.129$). For *B. grandis* a log linear model was fit to test for interaction between depth of burial, seed size and amount of dung. For the most parsimonious model (likelihood ratio $\chi^2 = 8.44$, $P = 0.586$) only the interaction seed size x burial depth was significant, which means for this species burial depth depends on seed size.

Controlled Germination Experiment

For all species examined, the proportion of seedlings emerging varied significantly with burial depth (for all Chi-square tests: $P < 0.01$). All species, except *M. guianensis*, showed a statistically significant linear trend, with a decreasing percentage of emerging seedlings with increasing burial depths (Fig. 4.6). Most buried seeds that did not emerge as seedlings germinated, but the seedling died before reaching the surface. In a few cases the buried seeds rotted.
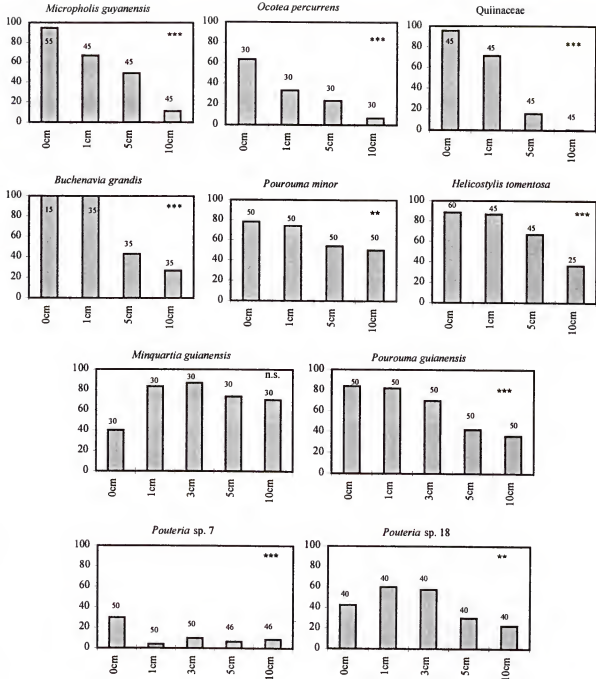
119

Figure 4.6. Percentage of seeds emerging as seedlings from different depths. Sample sizes are given in each bar. **, ***, and n.s. indicate P < 0.001, P < 0.0001, and P > 0.05, respectively, for Cochran's Test of linear trend.

The percentage of seedlings establishing from 5 cm and 10 cm, relative to the percentage of seedlings establishing at 1 cm (0 cm for *O. percurrens*) did not depend on seed size (linear regressions: for 5 cm, $F_{1,8} = 0.222$, $P = 0.89$, $r = 0.052$; for 10 cm, $F_{1,8} = 1.093$, $P = 0.33$, $r = 0.347$).

Discussion

Seed Burial by Dung Beetles

Between 26 and 67% of seeds (5-27 mm in length) in dung were buried by dung beetles at depths ranging from 0.5 to 20 cm. The percentages of seeds > 15 mm in length buried by beetles are generally higher than those reported by previous studies, but not for seeds ≤ 5 mm (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999). For example, seeds 18-20 mm in length were buried 12%, 13%, 20% and 25 % of the time in Peru, Mexico, French Guiana and Uganda, respectively. In this study I found that seeds in this size category were buried on average 40% of the time. These data all come from experiments in which dung beetles were allowed to naturally colonize dung piles, rather than experiments in which specific dung beetle species were tested for seed burial. The difference in results is in part due to differences in the dung beetle community. For example, dung beetle communities in the study sites in Mexico and Peru were less rich in species and individuals, and consisted of smaller dung beetles (Estrada and Coates-Estrada 1991, Estrada et al. 1993, Andresen 1999). No information on the composition of the dung beetle community is reported for the Ugandan site, and for the Guianan site information on only some species is given. Other possible sources of variation among studies include type of dung, freshness of the dung, amount of dung and

season of the year. Also, in the studies in Peru, Mexico and Uganda, an experimental setup was used in which the dung was placed on buckets or cylinders filled with soil, and dung beetles buried dung and seed inside the buckets. In my opinion this methodology can greatly affect the behavior of dung beetles, potentially yielding inaccurate results in terms of both percentage of seeds buried and depth of burial. For example, if beetles are confined to a small area, the encounter rate of competing beetles will propably be higher than under natural circumstances, and same dung beetles may chose to leave the dung pat.

In terms of burial depth, using buckets or cylinders may yield higher depths than would normally occur. For example, as pointed out by Vulinec (1999), since most beetles make tunnels at an angle from the dung pat (Halffter and Edmonds 1982), restricting them to a cylinder or bucket may force them to bury dung and seeds deeper than they normally would. Also, the soil filled into buckets is very likely to be less compacted and hard than soil under natural circumstances. Soil texture is known to affect both amount of dung buried and depth of burial (Fincher 1973, Davis 1996). Further, a dense mat of fine roots normally occurs under the soil surface in tropical forests, and this may affect beetle burial behavior.

Both the percentage of seeds buried and burial depth were related to seed size and amount of dung surrounding the seed. Smaller seeds were buried more often than larger seeds, and seeds surrounded by more dung were buried more often and more deeply than seeds surrounded by less dung. Medium-sized seeds (10 – 25 mm in length) were buried more deeply than either small or large seeds.

The relationship between seed size and proportion of seeds buried is in accordance with the findings of other studies (Estrada and Coates-Estrada 1991, Andresen 1999, Feer 1999) and makes intuitive sense. Seeds are dung "contaminants" for dung beetles and because beetles typically gather as much "pure" fecal material as possible, they often exclude seeds from the dung they bury. Thus, large seeds being larger contaminants than small seeds, are more often discarded from the dung portion the beetle is relocating for feeding or oviposition purposes. Why don't beetles remove all the seeds from the dung? Dung is a scarce and patchy resource and competition for it is great (Halffter and Edmonds 1982). Consequently, it is also advantageous for a dung beetle to relocate a portion of the fecal material as quickly as possible, thus leaving less time for removing unwanted seeds from the dung.

The non-linear relationship between seed size and burial depth can be due to seed characteristics such as shape, texture, density, or color, which influence the behavior of the dung beetles. In a different experiment, using plastic beads as seed mimics, I used bead weight rather than bead length as the independent variable, and found that the relationship between bead weight and burial depth was linear with a negative slope (Chapter 2). Shepherd and Chapman (1998) also found that smaller seeds were buried more deeply than larger seeds, but they only compared three species, ranging in size from 10 to 19 mm, a range that is within the medium-sized category in this study.

I found that seeds were buried more often and more deeply when they were surrounded by more dung than when they were surrounded by less dung. The cause for this relationship is most likely that larger dung piles attract more dung beetles than smaller dung piles (Peck and Howden 1984, Chapter 2). Consequently, competition may

be more intense in larger dung piles than in smaller ones. As already mentioned, intense competition may prompt the beetles to relocate dung portions quickly, not leaving much time for removing unwanted seeds. Similarly, a high number of beetles relocating dung may cause some beetles to bury it to greater depths, as a way of partitioning the vertical space below the dung source.

The relationships of the amount of dung surrounding the seed with the probability of the seed being buried by dung beetles and with the depth of burial has not previously been assessed. However, this relationship is likely of ecological importance because it links secondary seed dispersal (movement of seeds by dung beetles) to primary seed dispersal (defecation of seeds by arboreal mammals). Different species of frugivorous mammals have different defecation patterns (Chapter 3), characterized by the total amount of dung produced, the size distribution of fecal clumps, and the spatial distribution of fecal clumps. By knowing the type of defecation pattern typical of a given frugivore, one can assess the importance of secondary dispersal by dung beetles on the fate of seeds it disperses. This may be a useful variable when comparing the quality of different primary dispersers. Traditionally, different species of primary dispersers have been compared in terms of the number of seeds they disperse, the effect of passage through the animals gut, and the distance seeds are dispersed away from the parent plant (Schupp 1993). The effect of a frugivore's defecation pattern on the fate of dispersed seeds is usually not considered when assessing its effectiveness in seed dispersal (Schupp 1993).

Fate of Dispersed Seeds

This study has shown that seeds surrounded by fecal material produced the same proportion of seedlings as seeds dispersed without dung. This is because, even though seeds with dung that were buried produced more seedlings than seeds without dung on the soil surface, seeds with dung that remained on the surface suffered higher predation than seeds on the surface without dung. Also, some of the buried seeds suffered non-emergence of seedlings due to burial depth. When comparing the fate of seeds surrounded by dung, seeds buried by dung beetles had a much better chance of becoming seedlings than seeds on the surface.

Although smaller seeds and seeds surrounded by larger amounts of dung were buried more often by dung beetles, such seeds had no advantage in terms of seedlings produced, compared to larger seeds and seeds with less dung. This is because smaller seeds and seeds with more dung were buried more deeply, and this reduced the probability of both germination and seedling emergence. The controlled germination experiments showed very clearly that the seeds and germinating seeds of all species were negatively affected when burial depths increased. Studies on the germination and seedling establishment of temperate plant species have shown that an optimal range of burial depths exists for each species; while some seeds require very shallow burial depths, others do better when buried more deeply (Vander Wall 1993). Very few studies, however, have addressed the effect of burial depth on germination and seedling establishment of rainforest tree seeds. Two studies have at least indirectly assessed this issue for tropical species with small seeds and both found that seedling establishment decreased with depth (Fenner 1987, Dalling et al. 1994). Finally, a study in Uganda and

one in French Guiana, yielded similar results for a few large-seeded rainforest tree species (Shepherd and Chapman 1998, Feer 1999).

Interestingly, I found no relationship between seedling emergence from 5 and 10 cm depths and interspecific seed size. Previous studies on the seed dispersal role of dung beetles have speculated that plant species with larger seeds might be better able to emerge from greater depths than those with smaller seeds (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999) because of the larger energy reserves contained in larger seeds (Foster 1986, Westoby et al. 1992). This was not found in this study in a comparison of different species; however, the effect of burial depth for different-sized seeds at the intraspecific level remains to be investigated.

Seed characteristics other than size are probably important in determining the capacity of a germinating seedling to emerge from buried seeds. Plant species differ in the functional morphology of their seedlings, which in turn can largely determine the germination strategy adopted by a particular species (Garwood 1996, Kitajima 1996). From the results in this study, for example, it seems that seeds with fleshy cotyledons (reserve) were better able to emerge as seedlings from buried seeds, than seeds with foliaceous cotyledons (photosynthetic). Further, among seedlings with fleshy cotyledons, those that were phanerocotylar and epigeal emerged better than those that were cryptocotylar and hypogeal (see Methods for definitions of these terms).

In summary, although the overall net effect of dung beetles is positive when comparing the fate of buried seeds vs. surface seeds, burial by dung beetles also has a negative effect on seedling establishment due to decreased seedling emergence, and this

occurs for seeds of various sizes, not only small seeds as previously assumed (at least within the range of seed sizes used in this study).

Dung Beetles as a Selective Pressure on Seed Size

Shepherd and Chapman (1998) suggested that dung beetles could be acting as a selective agent on the evolution of seed size. They argued that if dung beetles consistently reduced the variability in seed survival by always depositing seeds in 'safer' sites, then they may be favoring directional selection of seed size, towards a size that increases the probability of burial by dung beetles. This would select for smaller seed sizes. On the other hand, if larger seeds (within a species) are better able to establish seedlings from buried seeds than smaller seeds, this should act also as a directional selection force, but in the opposite direction: selecting for larger seeds.

Alternatively, these two opposing effects on buried seeds: increased seed survival and decreased seedling emergence, could actually select in favor of increased seed size variation. Seed size variation as an adaptive strategy in plants due to differential advantages for small and large seeds has been proposed in other systems (Howe and Richter 1982, Silvertown 1989, Moegenburg 1996, Geritz 1998).

The trends observed in this study for the two species in which large and small seeds were used, suggest that dung beetles could be acting as a selective force on seed size. However, sample sizes were small, and the survival of one of the species was greatly affected by ants, which can act as a confounding variable. Further, these data do not help elucidate whether selection is favoring seed-size variation or whether it is stabilizing.

For *Pourouma minor*, small seeds were more often buried by dung beetles than large seeds, but small seeds were also buried more deeply than large seeds.

Unfortunately, sample sizes were not large enough to detect statistical differences. In terms of seedling establishment, large seeds buried by dung beetles tended to produce more seedlings than small buried seeds, when buried by dung beetles; again, the trend was not statistically significant.

In the case of *Buchenavia grandis*, data were not useful for testing if dung beetles are a selective force in seed size, because seed survival was greatly affected by ants. Small seeds of this species were also buried more often and more deeply than large seeds, and this difference was statistically significant. Also small seeds again had more difficulty emerging than large seeds when buried by dung beetles, but the differences were not statistically significant due probably to the effect of ants. Seeds of this species have an extremely hard exocarp that consists of two valves. When the seed is ready to germinate both valves begin to separate. At this stage of germination, leaf litter ants often invade the germinating seed, establishing a colony inside, and feeding on the cotyledons. This occurs in both buried and surface seeds. Thus, whereas for most other plant species, the advantage of burial by dung beetles lies in reduced predation by rodents, in the case of *B. grandis*, buried seeds are not effectively hidden from predation by ants.

Concluding Remarks

Although presence of dung and amount of dung surrounding dispersed seeds had an effect on the short-term fate of seeds, it did not have an effect on their long-term fate. The same proportion of seedlings emerged from seeds without dung and seeds with different amounts of dung. Thus, if one is comparing the fate of seeds of a plant species that has its seeds dispersed through different means (e.g., defecated by mammals vs. spit by mammals, or defecated by a mammal that produces small dung piles vs. one that

produces large dung piles) one could argue that the proportion of seedlings establishing from each dispersal mode will be the same. This will only be true if other aspects of each dispersal mode are also similar (e.g., seed treatment, site of seed deposition). Also, this will happen in a forest in which dung beetles are abundant and bury a large proportion of the seeds. In the case of a forest in which dung beetles are less abundant, are smaller, or seasonally absent, the long-term fate of seeds surrounded by dung may be very different from seeds dispersed without dung. In particular, seeds in dung may suffer increased predation from rodents and/or they may suffer increased fungus attack due to the fecal material not being removed completely by dung beetles (Jones 1994).

Also, this study focused on relatively large seeds (smallest seed size used in fate experiments was 11 mm in length). Results on long-term seed fate of seeds with dung vs. seeds without dung may be very different for very small seeds (< 3 mm). Most of these seeds will be buried by dung beetles when present in dung (Andresen 1999). Also, since small seeds are buried more deeply, they may only be able to establish as seedlings if they have a means of reaching the soil surface after some time. However many small seeds have long dormancy periods and may consequently not be affected negatively by seed burial. Such seeds may remain in the seed bank until brought up to the surface by other means. In this respect, dung beetles may also be important, as through their dung-burying activity they bring to the surface large quantities of soil from deeper strata (Hanski and Cambefort 1991b, Mittal 1993). On the other hand, very large seeds (> 40 mm) are unlikely to be buried by dung beetles (at least in the Neotropics), but dung beetles may still affect the fate of such seeds by removing the fecal material and thus reducing the odorous cue for seed predators.

Another limitation of this study is that it followed seed fate only until seedling establishment. It is possible that for some plant species, differences in seedling survival and/or growth may occur for seeds with and without dung, and for buried and unburied seeds. For example, due to their dung-burying behavior, dung beetles can significantly disturb the soil surface, which in turn can benefit seed germination and seedling survival/growth in several ways (Putz 1983).

In conclusion, this study described and quantified the effect of the dung beetle community on the fate of dispersed seeds in a rainforest in Central Amazonia. It has also shown, however, that each plant species responds in a slightly different way to the seed-dung beetle-seed predator interaction (Fig. 4.1, Table 4.4). Thus, in studies addressing the seed dispersal and seed fate of a species dispersed by arboreal mammals through defecation, it is important to perform experiments to assess the effect of the dung beetle community on the recruitment of that plant species. Such experiments should include one to assess the percentage of seeds that are buried by dung beetles, one to assess removal by seed predators of buried and surface seeds (with and without dung), and a controlled germination experiment to assess the effect of burial depth on seedling establishment. Also, because dung beetle activity affects plant species in slightly different ways, it can add to the variability in survival probabilities for different seeds within and among species. This variation in the risk of death can ultimately be an important force in promoting coexistence of plant species, allowing for the enormous diversity of plant species observed in tropical forests (Crawley 1992).

Finally, because dung beetles depend largely on the dung of large herbivorous mammals as a food source (Halffter and Edmonds 1982), and because most large

mammals are negatively affected by forest disturbance (Redford 1992), the regeneration of plant species dispersed through mammal defecation may be hindered through a demise of both their primary and their secondary dispersers, in disturbed habitats. This will be the focus of Chapter 5.

CHAPTER 5
EFFECT OF FOREST FRAGMENTATION ON DUNG BEETLES AND ON THE FATE OF SEEDS

Introduction

Tropical rainforests are the most diverse terrestrial ecosystem on earth. Although they represent less than 7% of the planet's land mass, they contain 50-75% of all species of plants and animals (Wilson 1988). Unfortunately, rainforests all over the world are disappearing at an alarming rate, with net tropical deforestation exceeding 150,000 km^2 per year (Whitmore 1997). Deforestation inevitably results in rainforest fragmentation, and both constitute great threats to biodiversity (Harris 1984, Wilson 1988, Laurance and Bierregaard 1997).

Fragmentation reduces biodiversity through at least four major mechanisms. First, because fragments represent a small sample of the original habitat, many species will disappear by chance alone when the fragments are created (random sampling effects). Second, fragmentation often prevents the normal movements and dispersal of animals and plants, which are crucial for long-term survival (isolation effects). Third, small fragments support smaller animal and plant populations, making them more susceptible to local extinction (area effects). Finally, biotic and abiotic influences from the surrounding landscape penetrate into fragments (edge effects), further reducing the area of suitable habitat (Harris 1984, Lovejoy et al. 1986, Skole and Tucker 1993, Noss and Csuti 1994).

Because the majority of the Amazon Basin will probably be subjected to development rather than being protected in large blocks (Bierregaard et al. 1992), it is most likely that the long-term conservation of this ecosystem will largely depend on well-planned management of fragmented forests. Such management will rely on a good understanding of the processes involved in natural forest regeneration and of the effects that forest fragmentation has on these processes.

Most studies on the effects of fragmentation have focused on first-order effects, i.e. physical changes and responses of individual species or group of species (Powell and Powell 1987, Malcolm 1988, Bierregaard and Lovejoy 1989, Kapos 1989, Schwarzkopf and Rylands 1989, Fronseca de Souza and Brown 1994, Brown and Hutchings 1997, Tocher et al. 1997, Turton and Freiburger 1997). These studies have shown that while some species are negatively affected by fragmentation, others are positively affected by it. Thus, generalizations about the effects of habitat fragmentation on species will be difficult at best, misleading at worst. And, while it is true that reserve planning and management require detailed studies on the ecology and effects of fragmentation on target species (Bierregaard et al. 1992), it is time that we adopt a more integrative approach. The study of plant-animal interactions and the associated process of plant regeneration offers one way of doing so.

Plant-animal interactions, such as pollination and seed dispersal, are thought to be important for the regeneration of tropical forests and for the maintenance of biodiversity; presumably, they have important implications for tropical conservation and management programs (Howe 1984, Pannell 1989, Terborgh 1990, Redford 1992, Chapman and Chapman 1995, Guariguata and Pinard 1998). And, although it is often stated that habitat

fragmentation likely disrupts plant-animal interactions, very few studies have actually addressed this issue (Renner 1998, and references therein). In this study I focus on seed dispersal -- one of the crucial stages in the life history of plants and, of course, important to forest regeneration (Harper 1977, Terborgh 1986a)

The study of seed dispersal is especially relevant in rainforests, because fruit-eating animals dominate the vertebrate biomass in such ecosystems (Terborgh 1986a), and because more than 75% of tree species in Neotropical forests produce fruits that appear adapted for animal consumption (Howe 1990). Thus, some rainforest plants may depend as much on their fruit consumers as vice versa (Bourliere 1985). Because fruit-eating animals appear to be especially sensitive to habitat disturbance (Johns and Skorupa 1987, Johns 1988, Symington 1988, Loiselle and Blake 1992, Redford 1992, Kattan et al. 1994, Levey 1994), studies on the interaction between rainforest plants and their animal seed dispersers in forest fragments are needed. The loss of a seed disperser may cause changes in the demography of plant species (Wright et al. 2000), which may in turn affect other animal populations, potentially leading to cascading effects (Howe 1984). Indeed, Redford (1992) has argued that a forest lacking frugivores may be just as doomed as a forest facing a chain saw. The threat of having forests in which ecological processes are not functioning normally, and which are likely to change drastically in the composition of their communities on the long-term, might be overlooked by studies that focus on the effects of fragmentation on a single species or group of species (e.g., trees), without linking such species to others and to ecosystem processes.

A crucial aspect in understanding the fruit-frugivore interaction, is the fate of dispersed seeds. Factors that affect the fate of dispersed seeds determine the degree to

which a plant's regeneration depends on dispersal by frugivores (Coates-Estrada and Estrada 1988). For example, plant species that are dispersed through seed ingestion and defecation by mammals, have most of their seeds deposited in fecal clumps, and the dung surrounding the seeds can have a large influence on the seeds' fate. In particular, the fecal material attracts dung beetles and rodents. Dung beetles, which bury dung for feeding and/or oviposition (Halffter and Edmonds 1982) also bury some of the seeds present in the dung (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999, Vulinec 1999). Like dung beetles, rodents are also attracted by the odor of fecal material and they actively remove seeds present in mammalian dung piles (Janzen 1982b, 1986). Unlike dung beetles, rodents feed on seeds. However, visual and/or olfactory detection of seeds by rodents is affected by seed burial, and seed predation rates are substantially lower for buried seeds than for seeds exposed on the surface (Johnson and Jorgensen 1981, Crawley 1992). Consequently, seeds buried by dung beetles have a high probability of avoiding detection by rodents (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999, Chapter 4).

In summary, a seed that is surrounded by dung due to dispersal through mammal defecation may have a very different fate than a seed of the same species that is dispersed without fecal material (e.g., spit-out or dropped by mammals, regurgitated by birds, etc.). Figure 5.1 shows some of the fates that seeds with and without dung may have after primary dispersal. The probabilities associated with each path are species-specific, and are likely to be affected by forest fragmentation.
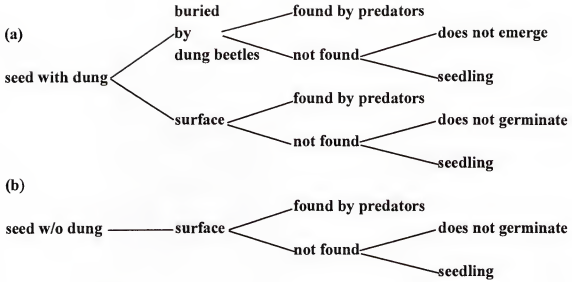
Fig. 5.1 Fate diagrams showing some of the paths that may be followed by seeds surrounded by dung (a) and seeds without dung (b). I am assuming that for the hypothetical seed species (as well as for the experimental species used in this study), scatterhoarding by rodents is not an important pathway. Seeds with dung may be buried by dung beetles or may remain on the surface, while seeds without dung are likely to remain on the surface. Both buried and surface seeds may be attacked by seed predators (rodents, insects, fungi, etc.) or may escape predation. Seeds attacked by seed predators are most likely killed. Surface seeds that escape predation are most likely to establish as seedlings. Buried seeds that escape predation will establish as seedlings unless the seeds do not germinate or burial depth is too deep for seedling emergence.

It is clear then, that if one wants to fully understand the dispersal ecology and regeneration requirements of a plant species, it is necessary to study the factors affecting the fate of seeds after seed deposition. If we cannot predict how secondary dispersers and seed predators affect the fates of seeds dispersed by other animals, then management plans focusing on primary dispersers might not reach their desired objectives.

The main objective of this study was to assess the effect of forest fragmentation on seed-dung beetle-seed predator interactions. Specific questions were:

(a) How do dung beetle communities in forest fragments compare to dung beetle communities in continuous forest?
(b) How do dung removal and secondary seed dispersal by dung beetles differ in forest fragments vs. continuous forest?
(c) How do seed predation rates differ in forest fragments vs. continuous forest?
(d) What are the fates of dispersed seeds in forest fragments compared to their fates in continuous forest?

Methods

Study Site

This study was conducted in five forest reserves that are part of the Biological Dynamics of Forest Fragments Project (BDFFP) and are located approximately 90 km north of the city of Manaus ($2^{o}30'S$, $60^{o}W$), in the Brazilian State of Amazonas (Fig. 5.2). One reserve is a 10,000 ha continuous forest site (reserve # 1501), and the other four are forest fragment reserves in the Colosso (CO) and Porto Alegre (PA) areas (Fig. 5.2). Two of the fragments consist of 1 ha of isolated forest (reserves # 1104 and # 3114, hereafter called 1-CO and 1-PA, respectively) and the other two are 10 ha of isolated forest (reserves # 1202 and # 3209, hereafter called 10-CO and 10-PA, respectively).

Reserves 1-CO and 10-CO were isolated in 1980 and reserves 1-PA and 10-PA in 1983 (Lovejoy et al. 1986). The fragments are almost perfect rectangles and the
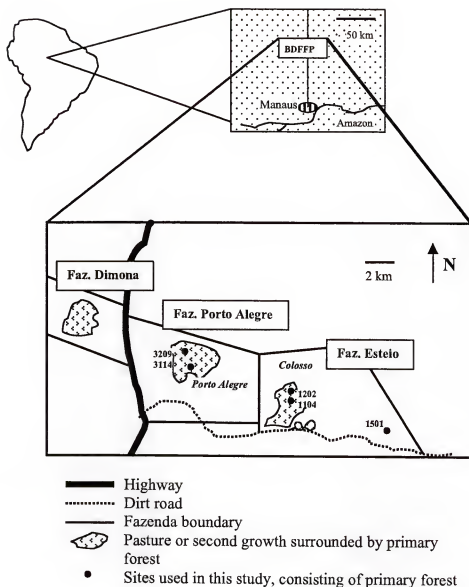
Figure 5.2 Location of the Biological Dynamics of Forest Fragments Project (BDFFP). Only the numbers of the reserves used in this study are shown. Reserves are located in three *fazendas* (cattle ranches), north of the city of Manaus. Reserves 1104 and 3114 are 1 ha forest fragments, reserves 1202 and 3209 are 10 ha forest fragments, and reserve 1501 is the continuous forest reserve.

vegetation surrounding them has varied through time and also among fragments due to differences in clearing methods (cutting, burning) and clearing frequencies (Bierregaard and Stouffer 1997). However, during the two years of my field research (May 1996 – May 1998), all four fragments were surrounded by pasture and/or a low (2-4 m tall) secondary growth vegetation composed mostly of *Vismia* spp. (Clusiaceae).

Mean annual temperature in Manaus is $26.7^{o}C$ and mean annual rainfall is 2,186 mm (Lovejoy and Bierregaard 1990), with a drier season between June and October. The area is mainly covered by dense *terra firme* forest (not subject to seasonal flooding) and continuous forest extends for hundreds of kilometers to the north, east and west (Bierregaard and Stouffer 1997). The forest is classified as tropical moist according to the Holdridge system (1967), with a 30-37 m tall canopy and emergents as high as 55 m (Lovejoy and Bierregaard 1990).

Since 1979, fragmentation has been studied in Central Amazonia by the BDFFP, which was formerly known as the Minimum Critical Size of Ecosystems Project (Lovejoy and Bierregaard 1990). The effects of forest fragmentation on microclimate and on specific groups of animals have been well documented by BDFFP (see Lovejoy et al. 1986, and references therein, Laurance and Bierregaard 1997). For example, it is known that only three out of six species of primates survive in fragments as small as 10 ha (Schwarzkopf and Rylands 1989), of which only the howler monkey (*Alouatta seniculus*) may be considered an important seed disperser for many plant species. It is also known that while populations of dung beetles decline in forest fragments (Klein 1989), rodent populations increase (Malcolm 1997), but show high turnover rates over time (Lovejoy et al. 1986).

Additional information about the study site and the BDFFP can be found elsewhere
(Lovejoy et al. 1986, Lovejoy and Bierregaard 1990, Rankin-de-Merona et al. 1992,
Bierregaard and Stouffer 1997).

The Dung Beetle Communities

To assess the effect of forest fragmentation on the dung beetle communities, I
captured dung beetles three times in the two 1 ha fragments (between August and
November 1997), five times in the two 10 ha fragments (between November 1996 and
September 1997) and five times in two transects in the continuous forest area (cont-E and
cont-W, 1 km apart, between November 1996 and September 1997). I trapped beetles in
1 ha fragments only three times because I decided later in my study to add these
fragments. Each trapping period in a given site occurred at least one month after the
previous. During each trapping period, beetles were captured at all sites within a two
month period to avoid having seasonality as a potentially confounding factor (Chapter 2).

Six pitfall traps were used in each of the trapping periods, in each site. Traps
consisted of plastic containers 15 cm high and 10 cm in diameter. Each trap was filled to
approximately two thirds of its capacity with soapy water (odorless soap was used) and
buried level with the ground surface. Fifty grams of fresh howler monkey dung was put
inside a bag made with plastic mosquito netting and suspended ca. 5 cm above the center
of the trap. Traps were placed in the forest, under intact canopy, at least 20 m away from
treefall gaps. The locations of the pitfall traps were the same during each trapping event
and traps were separated by at least 30 m. In the forest fragments traps were located
between 30 and 50 m from the forest edge. Traps were set out between 1600 and 1630 h
and dung beetles were collected the next morning between 0800 and 0830 h.

The dung used in the traps and in all experiments described below was usually collected in the morning (0700-1000 h), after I located a howler monkey troop near the research station. All seeds > 3 mm in the dung, were removed to avoid interference of these seeds with experimental seeds used in the experiments described below. Seeds ≤ 3 mm were not common in dung, their removal was not practical, and they were considered unlikely to affect the interaction between dung beetles and experimental seeds. The dung was kept in plastic bags in the shade and, since the freshness of dung is important for its attractiveness to dung beetles (Howden and Nealis 1975, Halffter and Edmonds 1982), it was generally used for trapping and/or experiments on the same day. When dung was used the next day, it was kept in an insulated box with ice. Dung was never stored for more than 36 h.

Dung beetles collected in the traps were stored in alcohol and taken to Manaus, where they were sorted by morphospecies. Samples of each morphospecies were pinned, dried and measured (body length). The mean size of beetles was calculated for each sample (six traps pooled for each trapping event), following Peck and Howden (1984). Mean size of the beetles in a sample is the summation of the number of each species multiplied by its mean length, divided by the total number of beetles caught. The size of beetles was considered important since it is the larger beetles that bury more and larger seeds, and are thus likely to be most relevant in terms of dispersal of seeds > 5 mm (Chapter 2). At the end of the study this collection was donated to the entomological collection of INPA (National Institute for Research in Amazonia), in Manaus. Other specimens were sent for identification to Fernando Z. Vaz-de-Mello (Setor de Ecologia,

Dep. de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36571-000, Brazil).

Dung Removal and Seed Burial by Dung Beetles

In experiments described in this section and in the section on Seed fate (see below) I did not use two transects in the continuous forest area (cont-E and cont-W, separated by 1 km), as I did for dung beetle trapping (previous section) and for experiments on Seed Predation (next section). Instead, I used several transects located in an area that encompassed both transects mentioned above.

To assess the effect of forest fragmentation on dung-removal and seed-burial by dung beetles, two experiments were conducted. In Experiment 1, dung removal and seed burial by beetles was compared in continuous forests vs. forest fragments. Because dung beetles in forest fragments are of smaller mean sizes than beetles in continuous forest (Klein 1989, this study), and because beetle size is important in determining whether a seed is buried (Feer 1999, Chapter 2), I hypothesized that seeds would be buried less often in fragments than in continuous forest, and if so, that mean beetle size would be a mechanism involved in producing this pattern. To test this, Experimet 2 manipulated the dung beetle community in continuous forest. Large dung beetles were excluded so as to mimic the dung beetle community in forest fragments, and seed-burial by small-medium sized beetles was compared to that of the entire dung beetle community (no beetles excluded).

Experiment 1: Dung removal and seed burial by beetles in continuous forest vs. forest fragments. To assess dung removal and seed burial by dung beetles in continuous forest vs. forest fragments I used single seeds inside 10 g dung piles. To be

able to find the seeds buried by dung beetles, a 50 cm-long white nylon thread was glued to each seed. I used one seed per dung pile to avoid nylon threads getting tangled. Also, in a previous experiment I had found that the same proportion of seeds of one seed species was buried by dung beetles in densities of 4 seeds/dung pile and 1 seed/dung pile (Chapter 3).

All dung piles were set out in the afternoon between 1500 h and 1800 h. I checked the transects after one day, recording dung removal (all dung removed vs. some dung remaining) and seed burial (seed buried by dung beetles vs. seed remaining on the surface). Seeds that had been buried by dung beetles were dug out and the depth of burial was measured to the nearest 1 cm (seeds slightly covered by soil were assigned a burial depth of 0.5 cm). Seeds that were not found and seeds in dung piles from which not all the dung had been removed were not included in the analyses of seed burial.

In this experiment I used seeds of three tree species: *Pourouma guianensis* (Moraceae; seed length: 11 ± 0.8 mm, N = 10), *Pouteria durlandii* (Sapotaceae; length: 27 ± 0.7 mm, N = 10) and *Micropholis guyanensis* (Sapotaceae; length: 18 ± 0.3 mm, N = 10; here and elsewhere I report means ± one standard error; hereafter plant species will be referred to by their generic name only). The first two species were used in the continuous forest and all four forest fragments, while *Micropholis* was only used in the continuous forest and the two 10 ha fragments because seeds of this species were not available when I added the 1 ha fragments. Data on percentage of seeds buried by beetles were pooled with data collected in Experiment 4 (see below). Since the type of seed in the dung pile was not relevant to the assessment of dung removal, data on dung removal were pooled for all three seed species. Additionally, these data were pooled with data on

dung removal gathered in Experiment 4 (see below; total sample sizes for each site are given in Fig. 5.5).

Experiment 2: Exclusion of large beetles in continuous forest. To compare the seed-burying behavior of the whole dung beetle community with that of an experimentally "impoverished" dung beetle community, I excluded large-sized beetles in the continuous forest, trying to mimic the situation of forest fragments. I put howler monkey dung (10 g) containing experimental seeds (see below) on the forest floor along transects, one pile every 10 m. I used two treatments: exclusion of large beetles and no exclusion. Treatments were alternated along the transects. To exclude the large beetles I placed a plastic mesh tray 30 cm long, 20 cm wide and 10 cm tall, upside down, above the seed-containing dung pile. The trays consisted of hard plastic mesh with mesh openings of 12 mm (to exclude the largest beetles, which are the most important for dispersal of seeds > 5 mm, Chapter 2). For the treatment without exclusion I put a 4 cm high plastic "fence", with the same length and width dimensions as the exclusion tray, around the dung pile. This prevented dung beetles from taking seeds outside this area, but still allowed them full access to dung piles.

I used the seeds of three tree species in this experiment: *Helicostylis scabra* (Moraceae; length: 5 ± 0.2 mm, N = 10), *Pourouma*, and *Pouteria*. In the 10 g dung piles I placed seeds of a single species, using 8 seeds in the case of *Helicostylis*, 4 seeds for *Pourouma* and 2 seeds for *Pouteria* (numbers of seeds were chosen so that approximately the same "seed volume" was present in each dung pile). Seeds had been dried in the sun (to avoid germination, deter rodent predation, and allow reuse of seeds) and painted with white paint (to facilitate detection and to deter rodent predation).

I set out all dung piles in the late afternoon, between 1500 and 1800 hours, to avoid having time of day as a confounding factor (see Chapter 2). Each location was checked after two days and the seeds remaining on the surface were counted. Sample sizes were 36 dung piles with exclusion and 36 without exclusion, for each of the three species.

To test if the plastic tray was effective in excluding large beetles, thus producing similar dung beetle assemblages as those found in forest fragments, I captured beetles in six pitfall traps, as described above, using the tray on top of the traps. The percentage of beetles captured with exclosure that were 10-15 mm in body length was 9, it was 1.3% for beetles 15-20 mm, and no beetles > 20 mm were captured. The percentage of beetles in these three size categories in the continuous forest (without exclosure) and in the forest fragments were as follows: beetles 10-15 mm: 9% in continuous forest, 1.4% in 10 ha fragments and 0.7% in 1 ha fragments; beetles 15-20 mm: 2.8% in continuous forest, 2.3% in 10 ha fragments and 0.2% in 1 ha fragments; beetles > 20 mm: 4.2% in continuous forest, 2.62% in 10 ha fragments and 0.43% in 1 ha fragments. Thus, while the exclusion treatment produced an "impoverished" dung beetle community with respect to the continuous forest, it did not mimic exactly the situation found in the 10 ha or 1 ha fragments studied.

Seed Predation

Experiment 3: Predation of sunflower seeds in continuous forest vs. forest fragments. To quantify relative seed predation pressure in continuous forest vs. forest fragments, I recorded predation on sunflower seeds. I used sunflower seeds because they could easily be bought in the market, they could be stored, and rodents are known to feed on them. Since I simply wanted to assess the relative predation pressures in each of the

sites, it did not matter what kind of seed I used as long as they were consumed by seed predators, so I chose a species that is likely to be palatable to most rodents. However, sunflower seeds are small and may also be removed by insect seed predators (e.g., ants), which could introduce a confounding variable (see Discussion).

I placed groups of five seeds on the forest floor along transects, one group every 10 m. Each station was checked after three days, when I recorded the number of seeds preyed (when seed husks were present) or removed (when no seed or seed husks could be found). Stations were placed in each of the four fragments and in two transects in the continuous forest, which were 1 km apart (cont-E and cont-W). In each of the six sites (two 1 ha fragments, two 10 ha fragments, and two continuous forest sites), 58-77 stations/site were placed during the dry season of 1997 and 36 stations/site were placed during the rainy season of 1998.

Seed Fates: Interaction of Seeds with Dung Beetles and Seed Predators

Experiment 4: Seed fates of three tree species in continuous forest vs. forest fragments. To determine whether the effects of forest fragmentation on dung beetle community composition and activity, and on seed predation pressures correlated with differences in seed fate, I followed the fate of seeds in forest fragments and continuous forest. I used two treatments: single clean seeds and single seeds inside 10 g dung piles. The no-dung treatment simulates seeds that have been spit out or dropped by mammals or regurgitated by birds. I used the same three seed species used in Experiment 1. For *Pourouma* and *Pouteria* I followed the fate of seeds in continuous forest and in the four fragments, while for *Micropholis* I followed the fate of seeds in the continuous forests and in the two 10 ha fragments. I could not conduct the experiment with *Micropholis* in

the 1 ha fragments because when I included these fragments, *Micropholis* was no longer fruiting.

I followed the same experimental setup as in Experiment 1, except that buried seeds were not dug out and they were checked after 1 day and then once a month until seed predation/removal, or seedling establishment. For each seed I recorded its fate (seed alive, seed eaten, seed removed, germinating seed, seedling) and location (buried or on the surface). A seedling was considered established when it had two leaves (either true leaves in the case of *Pourouma*, or cotyledons in the case of the other two species). When seeds had been removed, an effort was made to find the seed and/or thread; an area of approximately 5 m radius around the location was searched. Of the seeds removed by rodents and found, all were either eaten or hidden in a place that seemed unfavorable for seedling establishment (e.g., inside hollow logs). Seeds that were not found were assumed to be eaten by rodents.

I also recorded whether all dung had been removed after one day. These data were pooled with data in Experiment 1 to analyze dung removal by beetles. Data on seed burial were also pooled with data in Experiment 1 to assess overall seed burial by dung beetles for the three seed species. For each of the three species this experiment and Experiment 1 were conducted during the same months.

Data Analysis

Because I collected data in only two 1 ha fragments, two ten 10 ha fragments and one continuous forest area (with two independent transects, in the case of dung beetle captures and seed predation), I did not use these as individual samples, which would yield a sample size of just two for each treatment. Rather I use individual samples within sites

(e.g., five dung beetle trapping periods in 10-CO) for statistical analyses. This would result in pseudoreplication if I generalized my results to "all" continuous forests vs. 1-ha fragments vs. 10-ha fragments. To avoid this, my results will only apply to the sites I studied and the conclusions I reach will only be valid for them, and any generalization should be done keeping this limitation in mind.

The level of significance used in all tests was $\alpha = 0.05$ and all probabilities were adjusted when post-hoc comparisons were performed to maintain the same level of α for the whole set of comparisons. Bonferroni adjustments were used in case of ANOVA's (SPSS Inc. 1996), and in the case of Chi-square tests the adjustments shown in Sokal and Rohlf (1995) were used.

The dung beetle communities. I analyzed the dung beetle capture data using 2-level nested analyses of variance (ANOVA). I used four such analyses to test for differences in: number of individuals captured; number of species captured; mean size of beetles; and, percentage of individuals ≥ 10 mm long. The three treatments tested in the four nested ANOVA's were: continuous forest (two independent transects); 1-ha forest fragments (two sites); and, 10-ha forest fragments (two sites). To assess whether the differences found were due to treatments and/or to variation among the two sites in each treatment, I nested sites within treatments. Individual samples were the sampling events performed at each site: five in each continuous forest site and in each 10-ha fragment and three in each 1-ha forest fragment. The beetles collected in the six traps during each sampling event were pooled. Data on number of individuals were square-root transformed. Data on percentage of beetles ≥ 10 mm were transformed by using taking

the arcsin-square-root of proportions. Bonferroni adjusted post-hoc pairwise comparisons were performed for the four nested ANOVA's.

Dung removal and seed burial by dung beetles. Dung removal rates in the continuous forest and the fragments (Experiment 1) were compared on the basis of the frequencies of dung piles in which all dung was removed vs. dung piles in which not all dung was removed after one day, using Chi-square tests. The frequencies of seeds buried and not buried by beetles were compared in the same way. Adjusted probabilities were used for post-hoc pairwise comparisons between sites and the Cochran test for linear trends (SPSS Inc. 1996) was used to test for the increase in dung removal with increasing forest area. To compare the depths at which beetles bury seeds in continuous forest vs. fragments, a Kruskal-Wallis test was done for each seed species. Additionally, the frequencies of seeds buried < 5 cm deep and seeds buried $\geq$ 5 cm deep were analyzed with Chi-square tests.

For the experiment in the continuous forest in which large beetles were excluded and compared with the non-exclusion control (Experiment 2), the data were analyzed with a t-test for *Helicostylis* seeds and with a Mann-Whitney U-test for *Pourouma* and *Pouteria* seeds. For *Helicostylis* the proportion of the 8 seeds placed in each dung pile that was buried by beetles was calculated and the t-test was performed on arcsin-square-root of proportions. Four and two seeds were used per dung pile for *Pourouma* and *Pouteria* respectively, and no transformation yielded a normal distribution of the data; thus no parametric test could be performed for these species and the U-test was used instead. Additionally the data for *Pourouma* were pooled into two categories: 0-2 seeds

buried by beetles and 3-4 seeds buried and the frequencies were analyzed with a Chi-square test.

Seed predation. For Experiment 3, the frequency of seed stations with predation (1-5 seeds preyed/removed) was calculated for each site, for both the dry season of 1997 and the wet season of 1998. Comparisons within sites among both seasons and comparisons within seasons among sites were made with Chi-square tests.

Seed fate: interaction of seeds with dung beetles and seed predators. All comparisons performed in Experiment 4 were based on frequencies and analyzed with Chi-square tests or Fisher exact tests (in cases of low expected frequencies).

Results

The Dung Beetle Communities

A total of 14,608 beetles were captured, representing 58 species. Four species were captured in the 10 ha fragments that were not captured in the continuous forest. Three of these species were represented by only one individual. Five species were found solely in the continuous forest, and two of these were represented by one individual (Appendix B). In the 1 ha fragments, however, I captured only 28 species of dung beetles.

The same mean numbers of dung beetles per trapping period were captured in 1 ha fragments and in continuous forest sites. However, in the 10 ha forest fragments, dung beetles were twice as abundant (Fig 5.3 a). The numbers of beetles in the two sites within each treatment were found to be homogeneous (2-level nested ANOVA: treatment effect, $F_{2,3} = 9.69$, $P < 0.01$; effect of sites nested within treatments, $F_{3,20} = 0.48$, $P = 0.7$; post-

Figure 5.3 Mean number of individuals (a), mean number of species (b), mean size of beetles (c), and mean percentage of beetles ≥ 10 mm in length (d), captured in continuous forest (cont-E and cont-W), 10 ha forest fragments (10-CO and 10-PA) and 1 ha forest fragments (1-CO and 1-PA). Sample sizes are five trapping periods for continuous forest and 10 ha sites, and three trapping periods for 1 ha sites (in each period data of the six traps were pooled). Error bars represent ± 1 standard error.

hoc Bonferroni-adjusted pairwise comparisons among treatments: continuous vs. 10ha: P

< 0.01, 1ha vs. 10ha: P = 0.02, continuous vs. 1 ha: P = 1).

The mean numbers of species captured per trapping period were the same in

continuous forest and the 10 ha fragments, but was reduced by half in the 1 ha fragments

(Fig 5.3 b). Again, no differences were found among sites within treatments (2-level

nested ANOVA: treatment effect, $F_{2,3}$ = 36.30, P < 0.01; effect of sites nested within
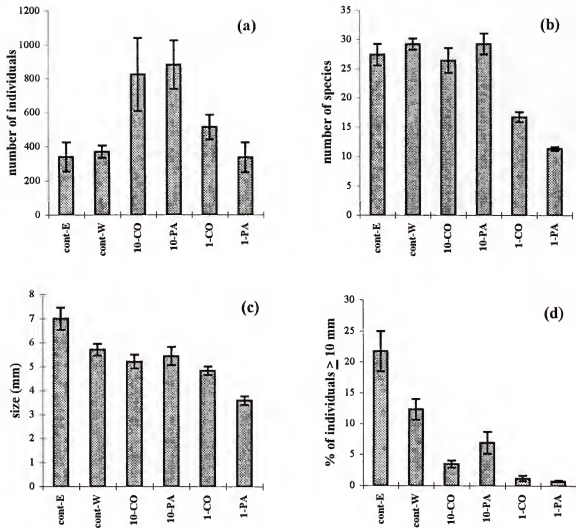
treatments, $F_{3,20}$ = 1.8, P = 0.17; post-hoc Bonferroni-adjusted pairwise comparisons

among treatments: continuous vs. 10ha: P = 1, 1ha vs. 10ha: P < 0.01, continuous vs. 1ha:

P < 0.01).

The mean size of dung beetles and the percentage of beetles ≥ 10 mm in length

were significantly higher in the continuous forest than in the 10 ha and 1 ha fragments

and they also were significantly higher in the 10 ha fragments than in the 1 ha fragments.

For both variables, variation among sites within each treatment was large and the effect

of sites nested within treatments was statistically significant (For mean size: 2-level

nested ANOVA: treatment effect, $F_{2,3}$ = 16.0, P < 0.01; effect of sites nested within

treatments, $F_{3,20}$ = 4.16, P = 0.02; post-hoc Bonferroni-adjusted pairwise comparisons

among treatments: continuous vs. 10ha: P = 0.01, 1ha vs. 10ha: P = 0.02, continuous vs.

1ha: P < 0.01. For percentage of beetles ≥ 10 mm: 2-level nested ANOVA: treatment

effect, $F_{2,3}$ = 58.28, P < 0.01; effect of sites nested within treatments, $F_{3,20}$ = 4.16, P =

0.01; post-hoc Bonferroni-adjusted pairwise comparisons among treatments: continuous

vs. 10ha: P < 0.01, 1ha vs. 10ha: P < 0.01, continuous vs. 1ha: P < 0.01; Figs. 5.3 c and
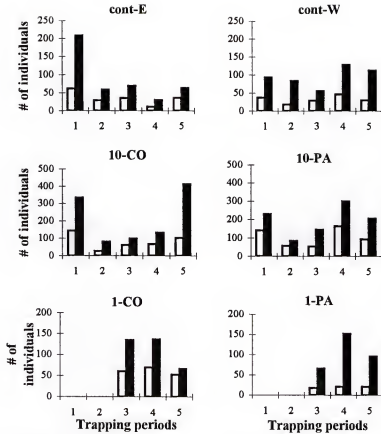
d).

Figure 5.4 Minimum (white bars) and maximum (black bars) number of individuals captured per trap in each trapping period (five for continuous forest and 10 ha fragments and three for 1 ha fragments). Site abbreviations are defined in Fig. 5.3.

Substantial variability was observed in the number of beetles captured both among the six traps used during a given trapping event and among trapping events (Fig. 5.4).

Dung Removal and Seed Burial by Dung Beetles

Experiment 1: Dung removal and seed burial by dung beetles in continuous forest vs. forest fragments. Dung removal by dung beetles was similar in continuous forest and 10 ha forest fragments, but the removal of dung was significantly slower in 1 ha forest fragments. While only 10% and 14% of dung piles in continuous forest and 10 ha fragments, respectively, had some dung remaining after 24 hours, 45% of dung piles in the 1 ha fragments had dung remaining after that period ($\chi^2 = 315.3$, d.f. = 2, P < 0.01; adjusted post-hoc pairwise comparisons: continuous vs. 10ha, P > 0.05; continuous vs. 1ha, P < 0.01, 10ha vs. 1ha, P < 0.01).

When individual sites were used in the comparison rather than pooling sites within treatments, the test was also highly significant ($\chi^2 = 359.20$, d.f. = 4, P < 0.01) and all adjusted post-hoc pairwise comparisons were significant (P < 0.01), except for continuous forest vs. the 10 ha fragment at the Porto Alegre area (P > 0.05; Fig. 5.5).

The percentage of seeds buried by beetles was always higher in the continuous forest than in the fragments (Fig. 5.6). This effect was statistically significant for *Pouteria* (overall $\chi^2 = 17.36$, d.f. = 4, P < 0.01) and *Micropholis* (overall $\chi^2 = 37.93$, d.f. = 2, P < 0.01), but not for *Pourouma* ($\chi^2 = 6.77$, d.f. = 4, P = 0.15). In the case of *Pouteria*, the continuous forest differed significantly from both Porto Alegre fragments (adjusted post-hoc comparisons, P's < 0.05), but not from the Colosso fragments (P's > 0.05) and all four fragments were homogeneous (P's > 0.05). For *Micropholis*, the
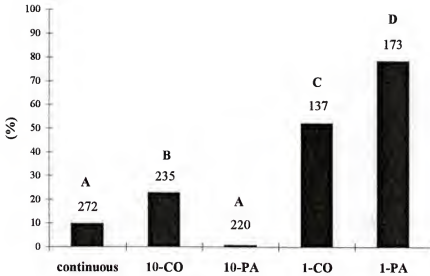
Figure 5.5 Percentage of dung piles with some dung remaining after 24 hours. Matching letters above bars identify statistically homogeneous samples (P > 0.05). Numbers of dung piles used are given above each bar. Site abbreviations are defined in Fig. 5.3.

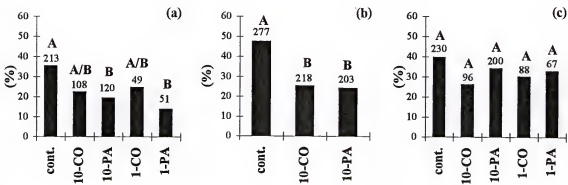Figure 5.6 Percentage of seeds of *Pouteria* (a), *Micropholis* (b) and *Pourouma* (c) buried by dung beetles in continuous forest and forest fragments. Matching letters above bars identify statistically homogeneous samples (P > 0.05). Number of seeds used are given above each bar. Site abbreviations are defined in Fig. 5.3.

continuous forest differed from both 10 ha fragments (adjusted post-hoc comparisons, P's < 0.05), but these two were homogeneous (adjusted post-hoc comparison, P > 0.05).

Regarding burial depths, seeds of the three species were buried more deeply in the continuous forest than in forest fragments (Fig. 5.7 a). However, the differences were only statistically significant for *Micropholis* in the comparison between the continuous forest and the Colosso fragment; the comparisons between continuous forest ant the Porto Alegre fragment, and between both fragments were not significant (Kruskal-Wallis = 9.13, P = 0.01, adjusted post-hoc pairwise comparisons: continuous vs. 10-CO, P < 0.05, continuous vs. 10-PA, P > 0.05, 10-CO vs. 10-PA, P > 0.05). The number of seeds of *Pouteria* buried in the 1 ha fragments was too small to allow analysis. No statistical differences were found in burial depth between continuous forest and the two 10 ha fragments for this species (Kruskal-Wallis = 4.66, P = 0.10). For *Pourouma*, the burial depth in all four fragments and the continuous forest was statistically homogeneous (Kruskal-Wallis = 4.45, P = 0.35).

The analyses on total frequencies of seeds buried < 5 cm deep and $\geq$ 5 cm deep yielded the same results as the analyses on burial depths (Fig. 5.7 b; *Micropholis*: χ^2 = 7.76, d.f. = 2, P = 0.02, adjusted pairwise comparisons: only continuous vs. 10-CO was significant [P < 0.05]; *Pouteria*: χ^2 = 1.72, d.f. = 2, P = 0.42; *Pourouma*: χ^2 = 1.59, d.f. = 4, P = 0.81).

Experiment 2: Exclusion of large beetles in continuous forest. For the three plant species used in this experiment, 13-19% fewer seeds were buried by dung beetles when large beetles were excluded than when they were not (Fig. 5.8). This difference was statistically significant for *Helicostylis* (t-test, T = 2.29, d.f. = 70, P = 0.03) and for
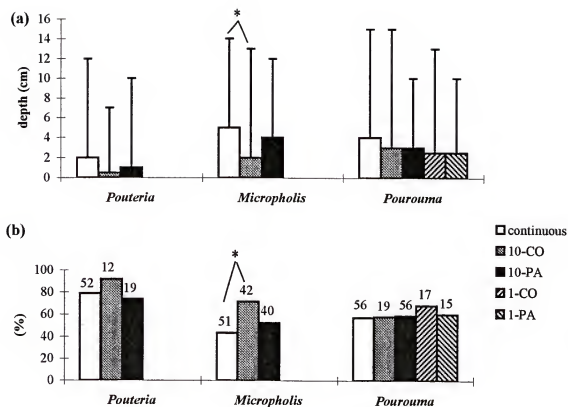
Figure 5.7 Median burial depth of seeds buried by dung beetles (a), and percentage of seeds buried less than 5 cm deep (b). Error bars in (a) indicate the maximum depth of burial; the minimum depth of burial was 0.5 cm in all cases. Sample sizes for both (a) and (b) are given in (b), above each bar. In both (a) and (b) only the comparison between continuous forest and 10-CO for *Micropholis* is statistically significant, as indicated by an asterisk (P < 0.05). Site abbreviations are defined in Fig. 5.3.
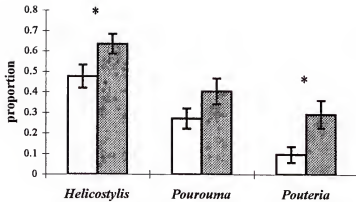
Figure 5.8 Proportion of seeds of three species buried by dung beetles when large dung beetles were excluded (white bars) and when no beetles were excluded (gray bars). N = 36 independent trials per species per treatment (exclusion vs. no exclusion of large beetles). Error bars represent ± 1 standard error. An asterisk indicates statistical significance, P < 0.05.

Pouteria (Mann-Whitney test, U = 806, N = 36 for both treatments, P = 0.02), but not for

Pourouma (Mann-Whitney test, U = 771, N = 36 for both treatments, P = 0.15).

When the data for *Pourouma* were pooled into two categories: 0-2 seeds buried and

3-4 seeds buried, a significantly lower frequency of 3-4 seeds buried occurred with the

exclusion or large beetles than without it ($\chi^2 = 4.43$, d.f. = 1, P = 0.04).

Seed Predation

Experiment 3: Predation of sunflower seeds in continuous forest vs. forest

fragments. No clear patterns in seed predation could be discerned from this experiment.

In the continuous forest and in the Colosso area, seed predation was higher during the wet

months of 1998 than during the dry months of 1997, while in the Porto Alegre area the

reverse occurred (Fig. 5.9). When the seasons were compared within each site, the

differences were statistically significant in the 10 ha fragment in Colosso (Fisher exact

test, P < 0.01) and the 1 ha fragment in Porto Alegre (Fisher exact test, P = 0.01). In the

other sites the differences were not significant (Fisher exact tests, P's > 0.05).

When sites were compared within season significant differences in seed predation

were observed (dry-97: $\chi^2 = 13.95$, d.f. = 5, P = 0.02; wet-98: $\chi^2 = 15.81$, d.f. = 5, P =

0.01). Adjusted post-hoc pairwise comparisons showed that in the dry months of 1997

five of the sites constituted a statistically homogeneous set (P > 0.05), while only the 1 ha

fragment in the Porto Alegre area had significantly more predation than the other sites (P

< 0.05). During the wet months of 1998 the Colosso fragments showed significantly

higher predation than the fragments in the Porto Alegre area (P < 0.05); one of the

continuous forest sites was statistically similar to all fragments (P > 0.05) while the other
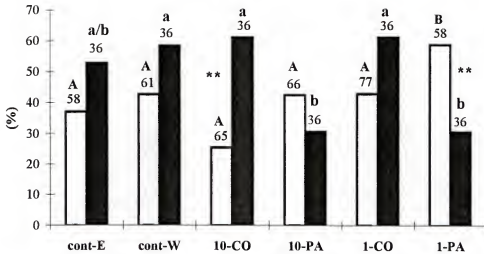
Figure 5.9 Percentage of stations with sunflower seeds that had seeds eaten or removed in the dry months of 1997 (white bars) and in the wet months of 1998 (black bars). Matching letters identify statistically homogeneous samples (P > 0.05): upper case letters for the dry months and lower case letters for the wet months. Number of stations used are given above each bar. ** denotes statistical significance in predation between dry and wet months (P < 0.01). Site abbreviations are defined in Fig. 5.3.

was similar to the Colosso fragments (P > 0.05) and showed significant higher predation than the Porto Alegre sites (P < 0.05; Fig. 5.9).

Seed Fates: Interaction of Seeds with Dung Beetles and Seed Predators

   Experiment 4: Seed fates of three tree species in continuous forest vs. forest fragments. The fates of dispersed seeds of *Micropholis*, *Pouteria*, and *Pourouma* are shown in Fig. 5.10. In this figure the fate of three types of seeds are given: (i) seeds placed on the forest floor without dung and consequently lying on the soil surface until predation/removal or seedling establishment (hereafter called "no-dung seeds"), (ii) seeds initially placed inside a dung pile, but not buried by dung beetles during dung removal (hereafter called "dung-surface seeds"), and (iii) seeds initially placed inside a dung pile and buried by dung beetles during dung removal (hereafter called "dung-buried seeds"). Seeds of types (ii) and (iii) pooled together constitute the "dung seeds". Fig. 5.14 gives the probabilities of seeds of the three species following different fate paths, in continuous forest and forest fragments.

   I present results for each species separately.

   Micropholis. When comparing the two original treatments, no-dung seeds and dung-seeds, no differences were found in the number of seedlings establishing in any of the sites (Fig. 5.11; continuous forest: $\chi^2 = 0.03$, d.f. = 1, P = 0.85; 10-CO: $\chi^2 = 0$, d.f. = 1, P = 1; 10-PA: $\chi^2 = 0.36$, d.f. = 1, P = 0.55; 10-CO+PA: $\chi^2 = 0.16$, d.f. = 1, P = 0.69; here and elsewhere CO+PA indicates the pooling of data from the Colosso and Porto Alegre areas).

   When comparing the three seed "states" existing after dung beetle activity (no-dung seeds, dung-surface seeds, and dung-buried seeds) the percentage of seedlings

Figure 5.10 Post-dispersal fate of seeds of three tree species, in continous forest, and forest fragments. nd-s are seeds placed on the forest floor with no dung which remain on the surface; d-s are seeds originally placed within a dung pile, but that also remained on the surface after dung removal by dung beetles; d-b are seeds originally placed within a dung pile, and that were buried by dung beetles during dung removal. Number of seeds are given above each bar. Site abbreviations are defined in Fig. 5.3; 10-CO+PA and 1-CO+PA represent the pooling of data for both 10 ha fragments and both 1 ha fragments, respectively.

Figure 5.11 Percentage of seedlings of three species establishing from no-dung seeds (white bars) and from dung seeds (grey bars) in continuous forest (cont.), in 10 ha fragments (two fragments pooled), and in 1 ha fragments (2 fragments pooled). Number of seeds are given above each bar; * denotes statistically significant difference, P < 0.05.
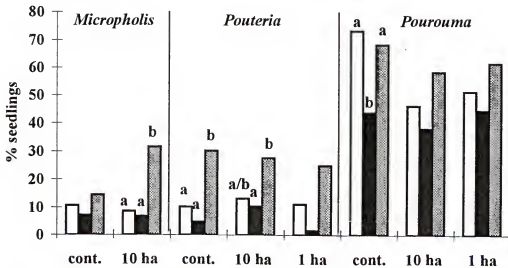

Figure 5.12 Percentage of seedlings of three plant species emerging from no-dung seeds (white bars), dung-surface seeds (black bars) and dung-buried seeds (grey bars). Site abbreviations are the same as in Fig. 5.11. Matching letters indicate statistically similar samples (P > 0.05); when no letters are present the three bars are statistically similar.

establishing was highest for dung-buried seeds, lower for no-dung seeds, and lowest for dung-surface seeds (Fig. 5.12). This was so in the continuous forest and the two 10 ha fragments, but the differences were only statistically significant in the Porto Alegre fragment and when pooling both fragments (continuous forest: $\chi^2 = 2.28$, d.f. = 2, P = 0.32; 10-CO: $\chi^2 = 3.83$, d.f. = 2, P = 0.15; 10-PA: $\chi^2 = 10.20$, d.f. = 2, P = 0.01; 10-CO+PA: $\chi^2 = 12.40$, d.f. = 2, P < 0.01). The adjusted post-hoc pairwise comparisons revealed that both in 10-PA and 10-CO+PA, significant differences occurred between no-dung seeds and the dung-buried seeds and between dung-surface seeds and dung-buried seeds, in both cases more seedlings established from dung-buried seeds (Chi-square tests, P's < 0.05). The percentage of seedlings establishing from no-dung seeds and dung-surface seeds was statistically similar (Chi-square tests, P's > 0.05).

When sites were compared in terms of the percentages of seeds preyed, analyzing separately no-dung seeds, dung-surface seeds, and dung-buried seeds, no significant differences were found, either comparing continuous forest with individual fragments or continuous forest with both fragments pooled (for all Chi-square and Fisher exact tests, P > 0.05). However, when all seed were considered together (no-dung seeds + dung seeds), a significantly lower percentage of seed predation was found in the continuous forest (75% predation) than in both fragments pooled together (87% predation; Fig. 5.13; $\chi^2 =$ 14.96, d.f. = 1, P < 0.01). When the individual fragments were used in the analysis, the test was also significant ($\chi^2 = 16.69$, d.f. = 2, P < 0.01) and adjusted post-hoc pairwise comparisons revealed that the significant differences occurred between the continuous forest and the Porto Alegre fragment (90% predation; $\chi^2 = 14.68$, d.f. = 1, P < 0.01), but
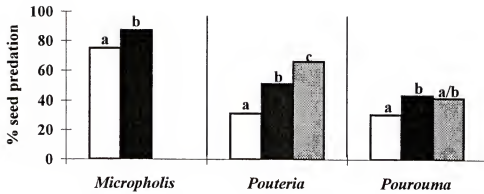
Figure 5.13 Percentage of seed predation for three plant species in continuous forest (white bars, in 10 ha fragments (black bars) and in 1 ha fragments (grey bars). Matching letters indicate, for each species, sites that are statistically similar (P > 0.05).

not between the continuous forest and the Colosso fragment (84% predation; $\chi^2 = 5.09$,

d.f. = 1, P > 0.05), or between fragments ($\chi^2 = 2.38$, d.f. = 1, P > 0.05).

Regarding the buried seeds that escaped predation/removal, but that did not emerge

as seedlings (most likely due to burial depth; see Chapter 4), the highest percentage

occurred in the continuous forest (81% of 57 seeds that were buried by beetles and not

preyed/removed by rodents), followed by 10-CO (72% of 11 seeds) and then by 10-PA

(50% of 6 seeds). However, the number of buried seeds in each site were too low to

detect statistical differences.

Pouteria. As with *Micropholis,* no differences were found in the number of

seedlings establishing in any of the sites, when comparing no-dung seeds and dung seeds

(Fig. 5.11; continuous forest: $\chi^2 = 0.56$, d.f. = 1, P = 0.46; 10-CO: $\chi^2 = 0.21$, d.f. = 1, P =

0.64; 10-PA: $\chi^2 = 0.07$, d.f. = 1, P = 0.80; 10-CO+PA: $\chi^2 = 0.03$, d.f. = 1, P = 0.86; 1-CO:

Fisher exact test, P = 1; 1-PA: Fisher exact test P = 0.06; 1-CO+PA: Fisher exact test, P =

0.21).

When comparing no-dung seeds, dung-surface seeds, and dung-buried seeds, the

percentage of seedlings establishing was, as with *Micropholis,* highest for dung-buried

seeds, lower for no-dung seeds, and lowest for dung-surface seeds, except in 1-PA. In 1-

PA only 2 seeds were buried by dung beetles, making this sample too small for reaching

reliable conclusions. A very high percentage of seeds of this species rotted (Fig. 5.10),

and as a consequence few seedlings established. Thus, for analysis, all surface seeds were

pooled (no-dung seeds + dung-surface seeds = surface seeds) and compared to the dung-

buried seeds. A significantly higher percentage of seedlings established from dung-buried

seeds than from surface seeds in the continuous forest ($\chi^2 = 20.77$, d.f. = 1, P < 0.01) and

in both Colosso fragments (10-CO: $\chi^2 = 4.22$, d.f. = 1, P = 0.04; 1-CO: Fisher exact test,

P = 0.03), but not in the Porto Alegre fragments (10-PA: Fisher exact test, P = 0.22, 1-

PA: Fisher exact test, P = 1).

Considering all seeds (no-dung seeds + dung seeds), a significantly lower

percentage of seed predation was found in the continuous forest (31% predation) than in

both 10 ha fragments pooled together (51% predation), and than in both 1 ha fragments

pooled together (66% predation; Fig. 5.13). Additionally, predation was significantly

lower in the 10 ha fragments pooled than in the 1 ha fragments pooled (overall $\chi^2 =$

49.84, d.f. = 2, P < 0.01; adjusted post-hoc comparisons: continuous vs. 10-CO+PA, P <

0.01; continuous vs. 1-CO+PA, P < 0.01; 10-CO+PA vs. 1-CO+PA, P < 0.01). When the

individual fragments were used in the analysis, the test was also significant (overall $\chi^2 =$

75.22, d.f. = 4, P < 0.01) and adjusted post-hoc pairwise comparisons revealed that

predation in the continuous forest was similar to predation in the Porto Alegre fragments

(P's > 0.05), but was significantly lower than predation in both Colosso fragments (P's <

0.01). In contrast, both 10 ha fragments did not differ significantly in seed predation (P >

0.05), but predation in the Colosso 1 ha fragment was significantly higher than predation

in the Porto Alegre 1 ha fragment (P < 0.01).

Due to the high proportion of rotten seeds and to the overall low proportion of seeds

buried by beetles, the frequency of non-emergence of seeds due to burial depth was

extremely low in this species and was not analyzed.

Pourouma. No differences were found in the number of seedlings establishing, in

any of the fragments, when comparing no-dung seeds and dung seeds (Fig. 5.11; 10-CO:

$\chi^2 = 2.89$, d.f. = 1, P = 0.09; 10-PA: $\chi^2 = 0.46$, d.f. = 1, P = 0.5; 10-CO+PA: $\chi^2 = 0.07$,

d.f. = 1, P = 0.79; 1-CO: χ^2 = 0.23, d.f. = 1, P = 0.64; 1-PA: χ^2 = 0.06, d.f. = 1, P = 0.81; 1-CO+PA: χ^2 = 0.03, d.f. = 1, P = 0.87). However, a difference existed in the continuous forest, in which significantly more seedlings established from no-dung seeds than from dung seeds (χ^2 = 8.80, d.f. = 1, P < 0.01).

When comparing no-dung seeds, dung-surface seeds, and dung-buried seeds in terms of the percentage of seedlings establishing, no statistically significant differences were found in the fragments (Chi-square tests, all P's > 0.05). However, in all fragments except in 10-CO, the same trend as with the other species was observed: the percentage of seedlings establishing was highest for dung buried seeds, lower for no-dung seeds, and lowest for dung-surface seeds. In 10-CO, percentage of seedlings was also highest for dung-buried seeds, but more seedlings emerged from the dung-surface seeds than from the no-dung seeds. In the case of the continuous forest, the highest percentage of establishing seedlings occurred for the no-dung seeds (Fig. 5.12). This percentage was significantly higher than the percentage of seedlings establishing from dung-surface seeds, but was not significantly different from the percentage of seedlings establishing from dung-buried seeds. Also, significantly less seedlings established from dung-surface seeds than from dung-buried seeds (overall χ^2 = 15.51, d.f. = 2, P < 0.01; adjusted post-hoc comparisons: no-dung seeds vs. dung-buried seeds, P > 0.05; dung-surface seeds vs. no-dung seeds, P < 0.01, dung-surface seeds vs. dung-buried seeds, P < 0.01).

When all surface seeds were pooled and compared to buried seeds, the only significant differences were found in 10-CO. In this fragment, significantly more seedlings established from buried seeds (43%), than from surface seeds (17%; χ^2 = 4.28, d.f. = 1, P = 0.04).
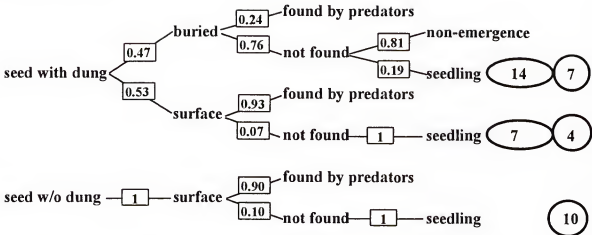
Micropholis
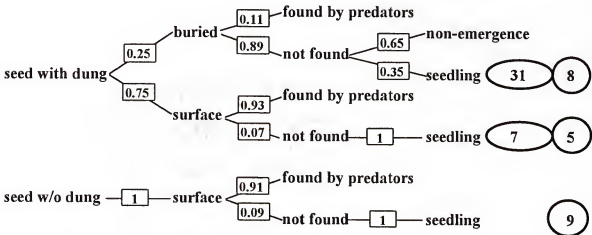
Figure 5.14 Fate of seeds surrounded by dung, and seeds without dung, in continuous forest and forest fragments. Numbers inside rectangles indicate the probability of a seed following a given path section. Numbers inside ovals are the number of seedlings establishing out of 100 seeds with dung that were buried by dung beetles and 100 seeds with dung that were not buried (second step of the path sequence), i.e., these numbers compare seedling establishment from dung-buried seeds vs. dung-surface seeds. Numbers inside circles are the number of seedlings establishing out of 100 seeds with dung and 100 seeds without dung (first step of the path sequence), i.e., these numbers compare seedling establishment from dung-buried seeds vs. dung-surface seeds vs. no-dung seeds.

Pourouma
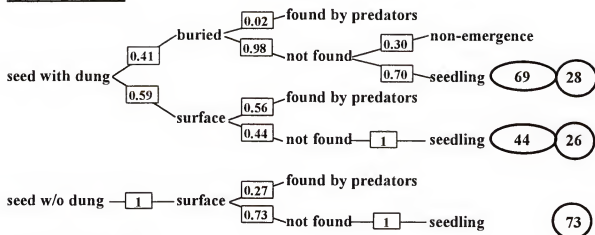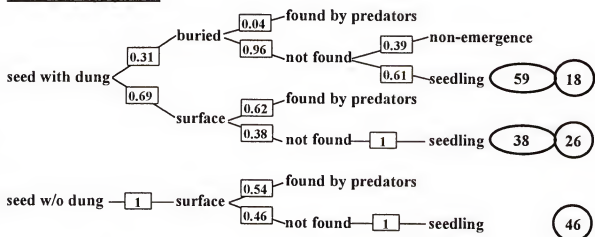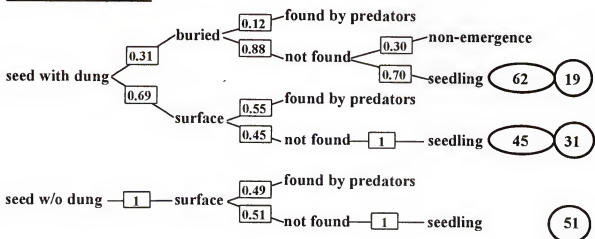
Figure 5.14 -- continued

Pouteria
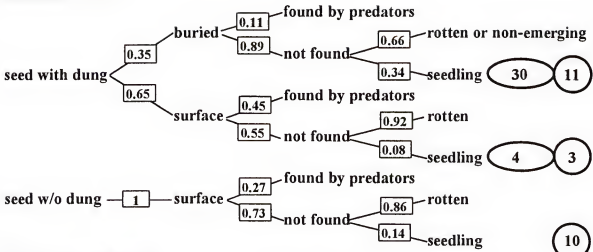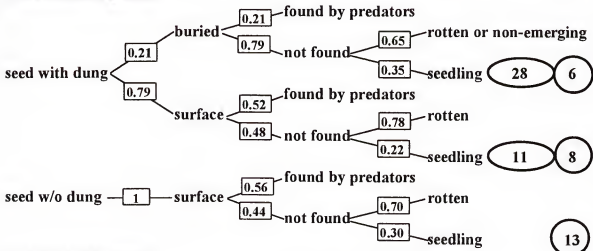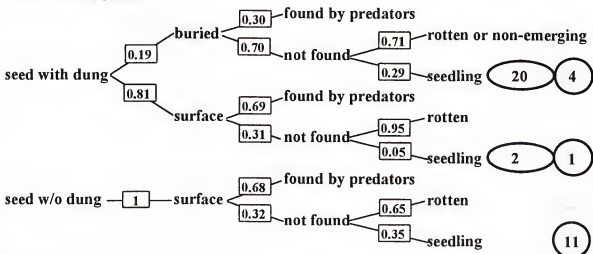
Figure 5.14 -- continued

Regarding seed predation, and again considering all seeds (no-dung seeds + dung seeds), a significantly lower percentage of seed predation was found in the continuous forest (31% predation), than in both 10 ha fragments pooled together (44% predation; Fig. 5.13). However, no significant differences were found in seed predation between the continuous forest and the 1 ha fragments pooled together (42%), or between 10 and 1 ha fragments (overall $\chi^2 = 8.67$, d.f. = 2, P = 0.01; adjusted post-hoc comparisons: continuous vs. 10-CO+PA, P < 0.05; continuous vs. 1-CO+PA, P > 0.05; 10-CO+PA vs. 1-CO+PA, P > 0.05). When the individual fragments were used in the analysis, the test was also significant (overall $\chi^2 = 33.65$, d.f. = 4, P < 0.01) and adjusted post-hoc pairwise comparisons revealed that predation in 10-CO was significantly higher than in all other sites (P's < 0.05), except 1-CO (P > 0.05). All other comparisons were not statistically significant (P's > 0.05).

Regarding non-emergence of buried seeds due to burial depth, the highest percentage of non-emerging seeds occurred in both Colosso fragments, was lower in the continuous forest and 10-PA and lowest in the 1-PA fragments. When all sites were compared, the analysis yielded results only suggestive of significance ($\chi^2 = 8.97$, d.f. = 4, P = 0.06).

In summary, although great variability was observed in the fate of seeds, among species, among sites, and with different species responding differently in the same sites, several patterns emerged. First, the same proportion of seedlings emerge from no-dung seeds than from dung seeds. Second, seeds buried by beetles have higher probabilities of establishing as a seedling than seeds remaining on the surface, and this advantage of

burial seems to be more pronounced in forest fragments. And third, seed predation rates tend to be higher in forest fragments than in continuous forest.

Discussion

The Dung Beetle Communities, Dung Removal and Seed Burial

The number of dung beetle species captured in the 10 ha fragments in Central Amazonia were equal to the number in continuous forest sites, but were double the number in the 1 ha fragments. Both the mean size of beetles and the frequency of large beetles ($\geq$ 10 mm in length) increased with increasing forest area. However, while the same number of individual dung beetles were captured in the 1 ha fragments and in the continuous forest sites, more than twice that amount was captured in the 10 ha fragments. In 10 ha forest fragments howler monkeys are able to survive, and the food resource available for dung beetles may not be seriously diminished. Furthermore, the home range of a howler monkey troop in the continuous forest is approximately 30 ha (Neves and Rylands 1991). Since the size of monkey troops is similar in fragments and continuous forest (personal observation), a monkey troop confined to a 10 ha fragment represents a much higher monkey density than one in continuous forest, and consequently produced more dung available per unit of area. This may be the reason for the much higher number of dung beetles captured in the 10 ha fragments vs. continuous forest. The availability of fresh mammal dung also seems to be affecting the populations of dung beetles in tropical forest fragments in Mexico (Estrada et al. 1998, 1999). In this site, researchers found that the abundance of dung beetle species and individuals was positively correlated with the number of non-flying mammals, and particularly to the number of howler monkeys.

My results are only partially in accord with the 1986 study conducted by Klein (1989) in the same study area (although not same sites). In the continuous forest, Klein captured more beetles, more individuals, and larger beetles than in both 10 ha and 1 ha fragments. The differences with the present study could in part be due to Klein's analyses, in which he pooled beetles captured with carrion and beetles captured with dung. So, for example, it could be that while more dung per unit area is available in the 10 ha fragments, the same in not true for carcasses. Further, when Klein conducted his study, the fragments had been isolated for only 2-6 years; it is probable that with time more beetle species disappeared from the 1 ha fragments, while the numbers of beetles started increasing in the 10 ha fragments. Also, Klein captured beetles at each site only once (a 4-day sampling period), making his sampling method susceptible to micro-temporal and micro-spatial fluctuations of the dung beetle assemblage (see below).

The rate of dung removal in the present study was similar in the continuous forest sites and in the 10 ha fragments, but was significantly lower in the 1 ha fragments. These results coincide with Klein's findings for the study area in 1986 (Klein 1989). The fact that dung removal was similar in continuous forest and 10 ha fragments, regardless of the much higher number of beetles captured in the 10 ha fragments, could be a consequence of the time elapsed before assessing dung removal. It is possible that if dung removal rates were measured after shorter periods of time (e.g., 6 hours instead of 24), a faster removal rate would be detected in the 10 ha fragments than in the continuous forest. If this is so, this might be an important difference between forest fragments and continuous forest, if seed predators are likely to find seeds soon after defecation. Under such circumstances it would be very advantageous to have a higher dung-removal rate in forest

fragments to balance the increased seed predation pressures found in these habitats (see below).

Alternatively, it could be that the higher frequency of beetles ≥ 10 mm in the continuous forest is responsible for a removal rate similar to that of the 10 ha fragments. Dung beetle size is positively correlated with the amount of dung consumed and dung burial rate (Lee and Peng 1981, Doube 1990). Difference in relative frequencies of large beetles is probably also responsible for the dung removal in the 1 ha fragments being significantly lower than in the other sites, even though the number of beetles captured was similar to the number captured in continuous forest. Since most dung beetles in Neotropical forests are probably attracted to fresh dung, rather than old dung (Howden and Nealis 1975, Halffter and Edmonds 1982, Peck and Forsyth 1982) and the small beetles of the 1 ha fragments were not able to remove all the dung quickly, the remaining dung often dried out, staying on the forest floor for long periods of time (> 1 month).

Larger dung beetles, probably because they bury larger dung portions, also bury more seeds present in the dung than do smaller beetles (Feer 1999, Vulinec 1999, Chapter 2). The importance of large beetles in secondary seed dispersal was confirmed by the results of Experiment 2, in which significantly lower proportions of three seed species were buried when large beetles were excluded in the continuous forest. The data on seed burial in fragments vs. continuous forest also point towards the relevance of beetle size in secondary seed dispersal. Fewer seeds in dung piles were buried by dung beetles in the forest fragments than in the continuous forest. The difference was statistically significant only for the two larger seed species (*Micropholis* and *Pouteria*), which stresses the particularly important role of large dung beetles in the secondary dispersal of large seeds.

It was surprising, however, to find no difference in the number of *Pouteria* seeds buried in the 10 ha vs. 1 ha fragments, since beetles in the 10 ha fragments were significantly larger and more abundant than in the 1 ha fragments. It may be that this very large seed (27 mm long), is mostly buried by the largest beetles. Since the frequency of beetles > 20 mm was very low in both 10 ha and 1 ha fragments (1% and 0.4% of all beetles captured, vs 4% in continuous forest), this may explain a similarly low proportion of buried seeds for *Pouteria* in 10 ha and 1 ha fragments.

In terms of burial depth, the data point towards a tendency of lesser burial depths in forest fragments than in continuous forest. However, most of the differences were not significant, due to small sample sizes and large variation. Burial depth is important in determining seed fate. On the one hand, seed detection by seed predators decreases with increasing depth (Estrada and Coates-Estrada 1991, Shepherd and Chapman 1998, Andresen 1999, Feer 1999). On the other hand, incidences of non-emergence of germinating seeds increase with increasing depth (Shepherd and Chapman 1998, Feer 1999, Chapter 4). Thus, it would be relevant to conduct additional experiments to test whether differences in burial depth between continuous forest and forest fragments are biologically important.

Variation in Dung Beetle Assemblage

Although general patterns of the dung beetle community and its activities in continuous vs. fragmented forest are discernible, it is important to keep in mind the variability that can exist between sites of similar habitat and area. Variability in the dung beetle communities among fragments or among continuous forest sites could be caused by factors associated with the site itself, such as edaphic or topographic differences.

Additionally, in forest fragments, the variability could be caused by factors related to the fragmentation process, such as different histories of the fragment's surrounding vegetation.

Variability exists not only among different sites, but also within sites. The average number of beetles captured in this study varied greatly between trapping events in each of the sites and also among traps within each trapping event (see Fig. 5.4). Temporal variability in the dung beetle assemblages do not seem to be strongly related to the seasonal pattern of rainfall (see Chapter 2), as is true in other tropical forests (Hanski 1980, Janzen 1983, Estrada and Coates-Estrada 1991, Gill 1991, Andresen 1999). Rather, the variation seems to be related to short-term local variables (e.g., weather conditions previous to the trapping event, amount of dung present in the area previous to the trapping event). Thus, it seems that much of the variation observed in dung removal and seed burial is probably due to micro-spatial and micro–temporal variability in dung beetle community composition. The micro-spatial variability is probably due to the aggregated spatial distribution typically found in insects that use patchy resources like dung (Hanski and Cambefort 1991d). The micro-temporal variability could be caused by short-term changes in temperature and/or rainfall. For example, abundance of beetles in a site in South Africa increased up to three fold following a day with 10 mm of rain (Doube 1991). Since most of the dung removal and seed burial occur during the first 24 hours, these events are likely to be affected by such short-term variability in the dung-beetle assemblage. Such within site variability can add to among site variability.

The differences observed between the two 1 ha fragments are probably the result of the Colosso fragment having a buffer zone around it, which is absent in the Porto Alegre

fragment. The buffer zone consists of an additional strip of forest (approx. 20 m wide) on each side of the fragment and contiguous with it. When the buffer zone is considered, the Colosso fragment is almost twice as large as the Porto Alegre fragment. Also, during the study period, a group of 3 howler monkeys inhabited the Colosso fragment (they were previously absent, Kellen Gilbert personal communication), while no monkeys were present in the Porto Alegre fragment. Howler monkeys are probably one of the most important sources of dung for dung beetles in many Neotropical forests (Howden and Young 1981, Peck and Forsyth 1982, Gill 1991, Estrada et al. 1999). Thus, it is perhaps not surprising to find that the Colosso fragment had more and larger beetles and that dung removal was faster, than in the Porto Alegre fragment.

All this variability within sites and among sites stresses the importance of being cautious when generalizing results from studies that were conducted in a few sites. Most studies on fragmentation suffer from this limitation, as it is virtually impossible to have several fragments of the exact same characteristics, which would allow for adequate replication. Consequently most of these studies, as is the case with the present study, use repeated sampling within each site, which leads to the results only being applicable to those sites. Also, forest fragments are dynamic and many processes and characteristics of these fragments are actively changing over time, making it again difficult to generalize (Kapos 1989, Kapos et al. 1997, Restrepo et al. 1999). Still, both authors and readers of fragmentation studies tend to generalize results over broader spatial and temporal scales. Surely, if such studies are to be applied to conservation and management programs, some generalization must be made at some point, but caution should be taken when doing so.

Seed Fate: Interaction of Seeds with Dung Beetles and Seed Predators

No clear pattern of the activity of seed predators was obtained from the experiment with sunflower seeds. Seed predation/removal varied greatly in space and time. High turnover rates of rodent populations have been thought to characterize the forest fragments in the study site (Lovejoy et al. 1986). Additionally, the activity of seed predators is strongly influenced by variables such as overall availability of food items and presence of preferred food items (Price and Jenkins 1986), which vary in both time and space in a given forest site. The lack of pattern in sunflower seed predation could be a consequence of this variability. Larger samples would be needed to detect any real differences among sites. Alternatively, it could be that other seed predators such as large ants also removed sunflower seeds, introducing a confounding variable.

Relative predation on sunflower seeds in the four fragments during the rainy season of 1998 was similar to that observed for *Pourouma* and *Pouteria* (which were also set out during the same season and year). For these two species, seed predation was 47% (*Pourouma*) and 41% (*Pouteria*) lower in the Porto Alegre fragments compared to the Colosso fragments. Similarly, predation on sunflower seeds was 49% lower in the Porto Alegre fragments than in the Colosso fragments. However, predation rates for *Pourouma* and *Pouteria* were in general higher in all four fragments than in the continuous forest, while sunflower seed predation in the continuous forest was similar to or higher than predation in the fragments. It may be that seed-removing insects are more abundant in the continuous forest than in the fragments, accounting for the higher predation rates observed for sunflower seeds.

A clearer pattern of seed predation was observed for the three native tree species used in the seed fate experiment, seeds of all of which are too large to be removed by insects. Predation on *Micropholis, Pourouma,* and *Pouteria* seeds was always higher in forest fragments (all fragments pooled) than in continuous forest. Additionally, predation of *Pouteria* seeds was significantly higher in the 1 ha fragments than in the 10 ha fragments. This result is consistent with Malcolm's (1997) findings of increased rodent populations in forest fragments, compared to continuous forest.

For the three seed species studied, and in all sites, the proportion of seedlings establishing from seeds with dung and from seeds without dung was the same. However, when comparing seeds that remained on the forest floor (either with or without dung) with seeds buried by dung beetles, a higher percentage of seedlings established from buried seeds than from surface seeds, in the continuous forest and forest fragments (all fragments pooled; see also Chapter 4). For *Pouteria*, the advantage conveyed by seed burial was statistically significant for both continuous forest and forest fragments. However, for the two other species, *Micropholis* and *Pourouma*, the difference in seedling establishment between surface seeds and buried seeds was only statistically significant in the forest fragments. These data suggest that while seed burial by dung beetles was beneficial for plant recruitment in all the forest sites I studied, it was more beneficial in forest fragments than in continuous forest. This is probably due to predation pressure on seeds, and particularly on surface seeds, being higher in the fragments than in continuous forest. Also, microclimatic conditions are often altered in forest fragments (Kapos et al. 1997) and buried seeds probably encounter a more homogeneous environment than surface seeds. In the continuous forest this effect may not be so

important, since microclimatic surface conditions probably fluctuate less. Finally, at least for *Micropholis*, data in this study suggest that seeds are buried less deeply in forest fragments, which decreases the proportion of germinating seeds that are not able to emerge due to burial depth.

Conservation Implications

Management for forest regeneration in forest fragments must be based on the most important components of plant dispersal and establishment. This study has shown that dung beetles and seed predators are an important part of dispersal biology of plants in tropical rainforests, and thus should be incorporated into dispersal studies and management programs.

The dung of mammalian herbivores is the most important source of food for dung beetles in most regions (Hanski 1991). Small forest fragments (10 ha or less) in Central Amazonia have greatly altered mammalian faunas. In particular, all large mammals, except howler monkeys, typically disappear from these fragments (Rylands and Keuroghlian 1988, Schwarzkopf and Rylands 1989). Depending on the distance of the fragment to continuous forest or to large forest fragments, other large mammals (e.g., other primates, deer, tapirs) can visit the small fragments periodically (personal observation). These sporadic visits by large mammals may represent an important source of food for dung beetles, particularly in small fragments where howler monkeys are not present. Also, these "visiting" mammals may be important in primary seed dispersal, and may even disperse seeds between fragments, which may help prevent inbreeding depression in plant populations (Nason et al. 1997). This argues in favor of maintaining the connectivity between forest fragments and continuous forest. Dung beetles are also

known to inhabit linear forest corridors, although they do not seem to use them to move between areas (Hill 1997). Further, for the corridors to have a similar dung beetle community as the one present in the continuous forest, they ought to have a minimum width of 200 m, which would also ensure the movement of many other animal species, including large vertebrates (Hill 1997).

While it is believed that dung beetle communities are mostly supported by howler monkeys in several Neotropical forests (Howden and Young 1981, Peck and Forsyth 1982, Gill 1991), it has also been reported that rich dung beetle communities can be found in forests in which howler monkeys and other large mammals are rare or absent (Peck and Forsyth 1982, and references therein). This suggests that beetles must also use the dung of other animals or other food items. In Mexico, however, researchers found that the number and biomass of beetles captured in forest fragments was positively related to howler monkey densities in those fragments, which in turn was negatively correlated to the area of the fragment (Estrada et al. 1999). This decrease in dung beetle populations in forest fragments may also be the consequence of other factors related to the fragmentation process, in addition to the impoverishment of the large-mammal fauna. The disappearance of some dung beetle species from small forest fragments could be caused by structural and microclimatic changes resulting from the fragmentation process. For example, the number of treefalls increases in forest fragments (Laurance et al. 1997) and most dung beetles in the Neotropics seem to avoid treefall gaps (Howden and Nealis 1975, personal observation). Additionally, very small fragments may have insufficient area to maintain long-term populations of some dung beetle species. This is further accentuated by the fact that the large majority of dung beetles do not cross clear-cut areas

(Klein 1989) and consequently are unlikely to recolonize isolated fragments after local extinction, unless small fragments were connected to larger fragments or to continuous forest.

Dung beetles play important roles in the seed dispersal ecology of plants that have their seeds dispersed by mammalian endozoochory. The probability of seedling establishment is increased when a seed is buried by dung beetles compared to when it remains on the surface. In my study sites, this effect seems to be more important in the forest fragments than in continuous forest, probably due to higher seed predation pressures in the fragments. Even the greatly altered dung beetle community of the 1 ha fragments could convey an important relative survival advantage to the seeds the beetles bury.

Since a seed needs to be surrounded by fecal material to be buried by dung beetles, and since only mammalian dung is likely to attract beetles that can bury medium-large seeds (Hanski and Cambefort 1991b), such seeds will probably be buried by beetles only when dispersed through mammal defecation. One of the main mammalian seed dispersers in many forest fragments in Central Amazonia is probably the howler monkey. Studies in Mexico and the Brazilian Atlantic forest have also pointed out the importance of howler monkeys as primary seed dispersers of many plant species in small forest fragments (Ferrari and Diego 1995, Estrada and Coates-Estrada 1996). Moreover, in the case of plant species that are likely to be dispersed only by mammals (due to fruit/seed morphology, e.g., *Micropholis* and *Pouteria*), the howler monkey may be the only seed disperser in forest fragments. Without dispersal away from the parent tree, most fruits/seeds falling underneath the parent's crown will likely suffer very high mortality

(Clark and Clark 1984). Such mortality may be even higher in some forest fragments, as some studies have reported increased densities of seed predators (rodents, insects, granivorous birds) in forest fragments and/or around forest edges (Laurance 1997, Malcolm 1997, Pizo 1997).

Unlike the fragments studied here, most forest fragments in tropical forest areas are subject to additional human disturbances such as poaching and logging. The degree of poaching on different species of animals varies greatly among countries, and also in different regions within countries (Peres 1990, 1997; Bodmer et al. 1997; Wright et al. 2000). Fortunately, although negatively affected by hunting pressures, howler monkey populations are very rarely decimated and seem to be less susceptible to hunting than other large-bodied primates (Peres 1990). Still, it would be recommendable to prohibit or regulate hunting in forest fragments, particularly if they are small and are likely to contain only one troop of howler monkeys.

It seems, then, that regeneration of many tree species in the forest fragments may be affected by the presence/absence of howler monkeys and other frugivorous mammals, and also by the presence/absence of a rich dung beetle community. If the regeneration of some plant species is affected, then the composition and structure of the plant community is likely to be affected over the long-run. A few studies in tropical rainforests have presented evidence supporting the fact that changes in the populations of animal species (seed dispersers and/or seed predators) can cause changes in the plant communities (Dirzo and Miranda 1991, Asquith et al. 1997, Chapman and Onderdonk 1998, Wright et al. 2000). However, these studies also show that many factors particular to each case, interact to ultimately determine which plant species are affected and what the direction of

the effect is. It follows, that while we can generalize that forest fragmentation and other forest disturbances (logging, poaching) will likely affect forest structure and composition, it is not possible to generalize as to what the specific net effect of such disturbance will be. This will have to be resolved empirically for each site (Wright et al. 2000).

CHAPTER 6
GENERAL CONCLUSIONS

The Dung Beetle Community and Secondary Seed Dispersal

The dung beetle community in my study site in Central Amazonia was rich in

species (61) and in number of individuals (120-706 individuals/10 traps in 12 hours).

Time of day and amount of dung affected on the number of individuals captured, with

more dung beetles captured at night and with larger baits. Time of day also had an effect

on mean beetle size, with larger beetles captured at night. Amount of dung also had an

effect on the number of species, with more species captured with more dung. Season of

the year had only an effect on the number of dung beetle species captured, with more

species captured during the rainy season.

Seed and bead (used as seed mimics) burial by dung beetles depended on seed or

bead size, beetle size, time of day, season, and amount of dung. Larger seeds, constituting

larger dung "contaminants" from the beetle's perspective, were buried less often and less

deeply than smaller seeds: on average $31 - 72$ % of small beads (mass < 0.5 g) were

buried at $2 - 6$ cm, and $6 - 30$ % of large beads (mass > 2 g) were buried at $1 - 4$ cm.

When individual dung beetle species were tested for their ability to bury seeds, beetle

size explained 87-92% of the variation in percentage of seeds buried for three seed

species, with larger beetle species burying more seeds than smaller beetles.

The effects of time of day and dung amount on the dung beetle assemblages apparently translated into an effect of these variables on seed burial by dung beetles. Seeds were buried more often and more deeply when placed at night and when surrounded by more dung. Season of the year had an effect on the dung beetle assemblage only in terms of number of species captured, which is likely less important in terms of seed burial. However, season of the year also affected secondary seed dispersal, with more seeds being buried during the wet season. It is probable that during the rainy season other factors, such as soil softness, facilitate seed burial by dung beetles.

Primary Seed Dispersal by Howler Monkeys and the Effect of Mammal Defecation Pattern on Short-Term Seed Fate

Howler monkeys (*Alouatta* spp.) studied in most other regions have been reported to disperse the seeds of less than 50 plant species. However, howlers in Central Amazonia dispersed the seeds of over 130 species. Also, in the study site, spider monkeys, the other species of large primate that is an important seed disperser, is very rare. Thus, for plant species that because of fruit and/or seed morphology can only be dispersed by arboreal mammals, howler monkeys are likely the single most important primary disperser in this forest.

Howler monkeys produced aggregated defecation with high dung densities and, depending on the proportion of fruit in the diet, also high densities of dispersed seeds. Also, individual dung piles were often of relatively large size (> 25 g). This contrasts with the more scattered spatial distribution of seeds and dung, and smaller individual dung piles, produced by most other rainforest mammals. When comparing rodent and dung beetle behavior in aggregated vs. scattered dung/seed patterns, I found that while

seed predation by rodents was higher in the aggregated dung/seed pattern, seed burial by dung beetles was the same in both patterns. Thus, while the size of an individual dung piles affects the probability of a seed being buried by dung beetles as well as the depth of burial (see above), the total amount of dung produced in a defecation does not affect secondary seed dispersal by dung beetles. Consequently, seeds defecated by mammal species that produce defecations that differ in their total amount of dung and/or seed densities, may have similar secondary dispersal by dung beetles, as long as the size distribution of individual fecal clumps is also similar.

In the case of howler monkeys, the high densities of dung produced may cause higher probabilities of seed predation by rodents, when compared to seed dispersal patterns produced by other mammals. However, this negative effect may be compensated for by the fact that individual dung piles of howler monkeys are probably also larger, on average, than those produced by other mammals. Consequently the probability of seed burial by dung beetles is higher for seeds present in howler monkey defecations, which in turn significantly decreases the probability of seed detection by rodents.

Seedling Establishment: Effects of Presence of Dung, Amount of Dung, and Seed Burial by Dung Beetles

Presence of dung had no effect on the long-term fate of seeds: for 10 out of 11 species, the same percentage of seedlings established from seeds with dung than from seeds without dung. However, when only the fate of seeds surrounded by dung was considered, seeds buried by dung beetles had a much better chance of becoming seedlings than seeds remaining on the surface.

Although smaller seeds and seeds surrounded by larger amounts of dung were buried more often by dung beetles, such seeds had no advantage in terms of seedlings produced, when compared to larger seeds and seeds with less dung. Smaller seeds and seeds with more dung were buried more deeply, which diminished the probability of seedling emergence. The controlled germination experiments showed that the seeds of all species were negatively affected by increasing burial depths. However, I found no relationship between seedling emergence from 5 and 10 cm of depth and interspecific seed size. Thus, although the overall net effect of seed burial by dung beetles was positive in terms of percentage of seedlings establishing when compared to unburied seeds, burial by dung beetles also had a negative effect due to increased emergence failure of buried seeds, and this occurred for seeds of all sizes, not only small seeds as previously assumed.

Effect of Forest Fragmentation on the Seed-Dung Beetle-Rodent Interaction

The 1 ha fragments had half the number of dung beetle species captured in continuous forest and in 10 ha fragments. The continuous forest sites and the 1 ha fragments had similar number of dung beetles, but in the 10 ha fragments dung beetles were twice as abundant. Howler monkeys are probably the most important dung source for beetles in 10 ha fragments, in which no other large mammals species survives long-term. Since the size of howler monkey troops is similar in fragments and continuous forest, and their home area in the continuous forest is approximately 30 ha, a monkey troop confined to a 10 ha fragment represents a much higher monkey density than that observed in continuous forest. This may be the reason for the much higher number of

dung beetles captured in the 10 ha fragments vs. continuous forest. Finally, mean size of beetles and the frequency of large beetles (>10 mm in length) increased with increasing forest area.

The differences in dung beetle communities, in particular the differences in mean size of beetles captured, translated into differences in dung removal and seed burial: both variables had higher values in continuous forest than in forest fragments. In terms of burial depth of seeds, a tendency for shallower burial depths in forest fragments was observed, but the results were not conclusive.

Seed predation for three tree species was higher in the forest fragments than in the continuous forest, which is consistent with the results of previous research on the small mammal communities in the study area. In particular, populations of some rodent species can be higher in forest fragments than in continuous forest.

For the three seed species studied the proportion of seedlings establishing from seeds with dung and from seeds without dung was the same, both in the continuous forest and in the forest fragments. However, when comparing seeds that remained on the forest floor (either with or without dung) with seeds buried by dung beetles, a higher percentage of seedlings established from buried seeds than from surface seeds, in all sites. For two of the species, the difference in seedling establishment between surface seeds and buried seeds was only statistically significant in the forest fragments. This suggests that while seed burial by dung beetles was beneficial for plant recruitment in all sites, it was relatively more beneficial in forest fragments than in continuous forest.

The maintenance of a rich dung beetle community in forest fragments may be closely related to the presence of howler monkeys. In turn, howler monkeys are probably

the main mammalian seed disperser capable of long-term survival in small forest fragments (*ca.* 10 ha) in Central Amazonia and other parts of the Neotropics. Moreover, for plant species that are exclusively dispersed by mammals (e.g. some large-seeded species, or fruits with hard indehiscent husks), the howler monkey may be the only seed disperser in forest fragments. Thus, the regeneration and long-term survival of many tree species in forest fragments may depend upon the presence of howler monkeys and may be enhanced by the presence of a rich dung beetle community, which in turn depends on the monkeys as a dung source.

APPENDIX A

DUNG BEETLES CAPTURED IN DIURNAL AND
NOCTURNAL TRAPPING PERIODS

Species of dung beetles captured in 14 diurnal and 14 nocturnal trapping periods
(10 traps/period, baited each with 25 g of fresh howler monkeys dung). The last
two columns classify the dung beetles according to their diel activity: d = mostly
diurnal, n = mostly nocturnal, and according to their dung processing behavior:
= tunnelers, R = rollers, D = dwellers. Mean body length, standard error, and
number of individuals measured are given in the first three columns. * indicates
a unique dung processing behavior in the genus *Eurysternus* in which dung balls
are only lightly covered by soil (Halffter and Edmonds 1982).

Species	Body length (mm)			# Individuals captured			Diel activity	Dung processing behavior
	mean	SE	N	day	night	total		
Aphodius sp. 1	3.5	0.13	8	3	200	203	n	D
Aphodius sp. 2	2.4	0.12	7	12	155	167	n	D
Ateuchus sp. 2	4.3	0.12	9	936	129	1065	d	T
Ateuchus sp. 3	2.9	0.11	5	227	30	257	d	T
Ateuchus sp. 4	4.4	0.08	6	4	2	6	?	T
Ateuchus sp. 5	6.5	0.10	10	12	316	328	n	T
Ateuchus sp. 6	6.7	0.09	8	9	194	203	n	T
Ateuchus sp. 7	7.7	0.10	6	9	7	16	?	T
Canthidium sp. 1	3.2	0.06	10	375	43	418	d	T
Canthidium sp. 2	6.4	0.24	8	50	2	52	d	T
Canthidium sp. 3	3.6	0.08	6	15	4	19	d	T
Canthidium sp. 4	4.6	0.11	6	30	849	879	n	T
Canthidium sp. 5	3.4	0.10	7	82	2	84	d	T
Canthidium sp. 6	5.5	0.14	10	243	21	264	d	T
Canthidium sp. 7	4.8	0.12	10	416	37	453	d	T
Canthidium sp. 8	7.4	0.26	10	0	345	345	n	T
Canthidium sp. 9	7.7	0.22	9	0	34	34	n	T
Canthon sordidum (Har.)	6.2	0.14	10	11	0	11	d	R
Canthon sp. 1	6.5		1	2	0	2	?	R
Canthon sp. 2	5.1	0.15	9	73	5	78	d	R
Canthon sp. 3	6.6	0.19	10	34	0	34	d	R
Coprophanaeus lancifer (L.)	38.4	0.91	10	0	1	1	?	T
Cryptocanthon peckorum (Howden)	2.3	0.10	5	7	22	29	n	R
Deltochilum orbiculare Lansberge	24.8	0.61	10	1	28	29	n	R
Deltochilum sp. 2	24.3	0.55	9	0	9	9	n	R
Deltochilum sp. 3	12.4	0.19	10	0	27	27	n	R
Dichotomius boreus (Oliv.)	24.7	0.50	10	0	254	254	n	T
Dichotomius lucasi (Luederwaldt)	12.1	0.24	9	3	629	632	n	T

Appendix A -- continued

Species	Body length (mm)			# Individuals captured			Diel activity	Dung processing behavior
	mean	SE	N	day	night	total		
Dichotomius sp. 1	16.1	0.34	7	0	44	44	n	T
Dichotomius sp. 2	15.3	0.24	8	0	25	25	n	T
Dichotomius subaeneus (Laporte)	16.6	0.50	10	0	38	38	n	T
Eurysternus caribaeus (Herbst)	14.1	0.44	10	18	20	38	d/n	*
Eurysternus hirtellus Dalman	6.1	0.29	10	65	0	65	d	*
Eurysternus velutinus Bates	16.9	0.30	10	7	2	9	?	*
Neocanthidium atricolle (Preudh.)	8.9	0.30	10	49	1	50	d	T
Neocanthidium auricolle (Har.)	8.1	0.17	10	27	5	32	d	T
Neocanthidium sp.1	4.9		1	7	0	7	d	T
Ontherus carinifrons Luederwaldt	12.4	0.45	10	0	51	51	n	T
Onthophagus bidentatus Drapiez	5.3	0.20	10	146	48	194	d	T
Onthophagus sp. 2	5.0		1	31	3	34	d	T
Oxysternon durantoni Arnaud	14.6	0.35	8	85	0	85	d	T
Oxysternon festivum d'Olsovfieff	20.1	0.48	6	27	0	27	d	T
Oxysternon prox. *silenum*	15.2	0.45	10	52	1	53	d	T
Phanaeus chalcomelas Perty	13.5	0.27	8	54	0	54	d	T
Scybalocanthon pygidialis (Schmidt)	8.4	0.11	10	87	10	97	d	R
Scybalocanthon sp. 1	9.5	0.21	10	75	3	78	d	R
Uroxys pigmaeus Har.	2.7	0.10	10	81	1655	1736	n	T
sp. AA	2.8	0.60	2	5	2	7	?	?
sp. DD	10.2	0.21	5	0	1	1	?	?
sp. EE	2.2		1	0	1	1	?	?
sp. FF	14.3	0.39	6	0	4	4	n	?
sp. GG	6.3	0.12	10	0	25	25	n	?
sp. H	3.5	0.32	4	5	0	5	d	?
sp. HH	4.3	0.10	2	0	2	2	?	?
sp. JJ	9.8		1	0	1	1	?	?
sp. KK	4.4	0.15	9	0	7	7	n	?
sp. O	2.0		1	9	9	18	d/n	?
sp. P	1.6	0.10	2	28	6	34	d	?
sp. R	6.2	0.22	6	0	7	7	n	?
sp. W	2.7	0.10	10	11	4	15	d	?
sp. Y	1.5		1	101	1	102	d	?

APPENDIX B

DUNG BEETLES CAPTURED IN CONTINUOUS
FOREST AND FOREST FRAGMENTS

Total number of dung beetles captured in five trapping periods in continuous
forest sites (cont-E and cont-W), in five trapping periods in 10 ha forest
fragments (10-CO and 10-PA, and in three trapping periods in 1 ha forest
fragments (1-CO and 1-PA). Six traps were used in each trapping period, each
baited with 50 g of fresh howler monkey dung. Mean body length of dung beetle
species, standard error, and number of beetles used to calculate mean body
length (N) are also given.

| Species | Body length (mm) | | | # individuals captured | | | | | |
	mean	SE	N	cont-E	cont-W	10-CO	10-PA	1-CO	1-PA
Aphodius sp. 1	3.5	0.13	8	37	65	52	151	2	7
Aphodius sp. 2	2.4	0.12	7	35	56	114	137	117	4
Ateuchus sp. 2	4.3	0.12	9	221	347	707	733	83	32
Ateuchus sp. 3	2.9	0.11	5	9	24	24	28	1	2
Ateuchus sp. 4	4.4	0.08	6	9	0	1	1	0	0
Ateuchus sp. 5	6.5	0.10	10	16	33	12	18	3	0
Ateuchus sp. 6	6.7	0.09	8	41	40	14	212	7	6
Ateuchus sp. 7	7.7	0.10	6	12	3	0	12	0	0
Canthidium sp. 1	3.2	0.06	10	4	38	31	30	8	1
Canthidium sp. 2	6.4	0.24	8	1	1	1	1	0	0
Canthidium sp. 3	3.6	0.08	6	2	5	0	0	0	0
Canthidium sp. 4	4.6	0.11	6	142	290	793	589	57	1
Canthidium sp. 5	3.4	0.10	7	0	1	1	0	0	0
Canthidium sp. 6	5.5	0.14	10	1	0	0	0	0	0
Canthidium sp. 7	4.8	0.12	10	157	88	298	187	63	50
Canthidium sp. 8	7.4	0.26	10	48	73	63	109	3	42
Canthidium sp. 9	7.7	0.22	9	4	9	6	11	0	1
Canthon sordidum (Har.)	6.2	0.14	10	0	2	1	23	0	0
Canthon sp. 2	5.1	0.15	9	1	3	12	3	0	0
Canthon sp. 3	6.6	0.19	10	0	2	2	6	0	0
Coprophanaeus lancifer (L.)	38.4	0.91	10	0	1	2	2	1	0
Coprophanaeus sp. 1	30.8		1	0	0	1	0	0	0

Appendix B -- continued

Species	Body length (mm)			# individuals captured					
	mean	SE	N	cont-E	cont-W	10-CO	10-PA	1-CO	1-PA
Cryptocanthon peckorum (Howden)	2.3	0.10	5	0	9	1	0	0	0
Deltochilum orbiculare Lansberge	24.8	0.61	10	19	6	0	4	0	0
Deltochilum sp. 2	24.3	0.55	9	4	2	1	1	0	0
Deltochilum sp. 3	12.4	0.19	10	18	23	0	2	1	0
Dichotomius boreus (Oliv.)	24.7	0.50	10	72	43	18	85	3	6
Dichotomius lucasi (Luederwaldt)	12.1	0.24	9	126	78	8	35	1	1
Dichotomius sp. 1	16.1	0.34	7	12	10	20	28	0	0
Dichotomius sp. 2	15.3	0.24	8	15	8	32	17	1	0
Dichotomius subaeneus (Laporte)	16.6	0.50	10	9	15	41	47	4	0
Eurysternus caribaeus (Herbst)	14.1	0.44	10	32	26	6	44	4	0
Eurysternus hirtellus Dalman	6.1	0.29	10	1	4	0	8	2	0
Eurysternus velutinus Bates	16.9	0.30	10	6	14	2	5	0	0
Neocanthidium atricolle (Preudh.)	8.9	0.30	10	2	1	1	0	0	0
Neocanthidium auricolle (Har.)	8.1	0.17	10	3	0	0	1	0	0
Ontherus carinifrons Luederwaldt	12.4	0.45	10	1	1	6	0	0	0
Onthophagus bidentatus Drapiez	5.3	0.20	10	29	78	925	827	974	97
Onthophagus sp. 2	5.0		1	0	0	15	0	0	0
Oxysternon durantoni Arnaud	14.6	0.35	8	6	2	1	0	0	0
Oxysternon prox. *silenum*	15.2	0.45	10	3	0	3	1	0	0
Phanaeus chalcomelas Perty	13.5	0.27	8	1	0	1	4	0	0
Scybalocanthon pygidialis (Schmidt)	8.4	0.11	10	33	15	25	13	0	0
Scybalocanthon sp. 1	9.5	0.21	10	34	6	215	59	4	8
Uroxys pigmaeus Har.	2.7	0.10	10	507	392	568	908	179	746
sp. DD	10.2	0.21	5	0	1	2	11	3	0
sp. FF	14.3	0.39	6	5	1	0	0	0	0
sp. GG	6.3	0.12	10	1	4	0	0	0	1
sp. H	3.5	0.32	4	1	3	12	3	0	0
sp. HH	4.3	0.10	2	0	0	0	1	0	0
sp. JJ	9.8		1	0	1	0	0	0	0
sp. KK	4.4	0.15	9	0	3	9	25	4	0
sp. O	2.0		1	3	3	0	0	0	0
sp. P	1.6	0.10	2	3	12	22	1	2	2
sp. R	6.2	0.22	6	0	1	1	1	0	0
sp. W	2.7	0.10	10	12	6	30	8	2	1
sp. Y	1.5		1	0	2	13	9	7	0
sp. CC	2.5		1	0	0	0	1	0	0

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BIOGRAPHICAL SKETCH

Ellen Andresen received a bachelor's degree and the title of biologist from the Universidad Nacional Agraria "La Molina," in Lima-Perú, in 1990. She received an MS from Duke University, School of the Environment, in 1994.

Note. For the benefit of those readers who are curious enough to reach this section, and actually read it with the hopeful anticipation of finding something "interesting" (which, of course, is a term as vague as each person's anticipation), I shall include some facts that might, or might not, throw some insight into the intrinsic causes of this dissertation.

1968: despite my early rebellious inclinations my parents insisted on baptizing me. While in church, and while being carried by my very loving, but also very respectful and very German grandmother, I decided to clearly express my opinion. As I was unable to speak yet, and realizing that my crying efforts did not help me much. I took a less conventional course of action…much for the entertainment of some and the despair of others.

1969: being less than a year old, and being pushed in my carriage by a very playful but not-so-young great-aunt, who liked to push the thing (with me inside) ahead of her, and then run after it, I fell out of my carriage. My dear great-aunt had not realized the extent of the street's slope, and had failed to catch the carriage before it came to a more,

let's say, "natural" stop. Although she reported no obvious injury, doubts may arise, and this seemingly funny and innocent incident might, in retrospect, help explain many things.

1972: my first year of kindergarten: I refused for a whole year to talk or play with any of the kids. Somehow, I had prematurely realized that it is often easier to interact with animals and plants, than with people. However, during my second year of kindergarten, and after having sensed an unusual degree of preoccupation in my mother, I gathered all my courage and started playing with children. I even started enjoying the company of some.

1977: second attempt to save my soul: I had my first communion. I liked my dress, the little flowers on my head, and even the cross pendant, but could not make much sense out of anything else. A few years after that I gave up mysticism and found true enlightenment in atheism and biology.

1990: after growing up in Lima (where storms are non-existent and people don't own umbrellas), I saw a lightning bolt for the first time in my life, while canoeing in an oxbow lake during my first visit to the rainforest. I realized I had come to the right place.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Douglas Levey, Chairman
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard Bodmer
Assistant Professor of Wildlife
    Ecology and Conservation

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.
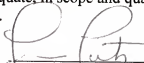
H. Jane Brockmann
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Colin Chapman
Associate Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 2000

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